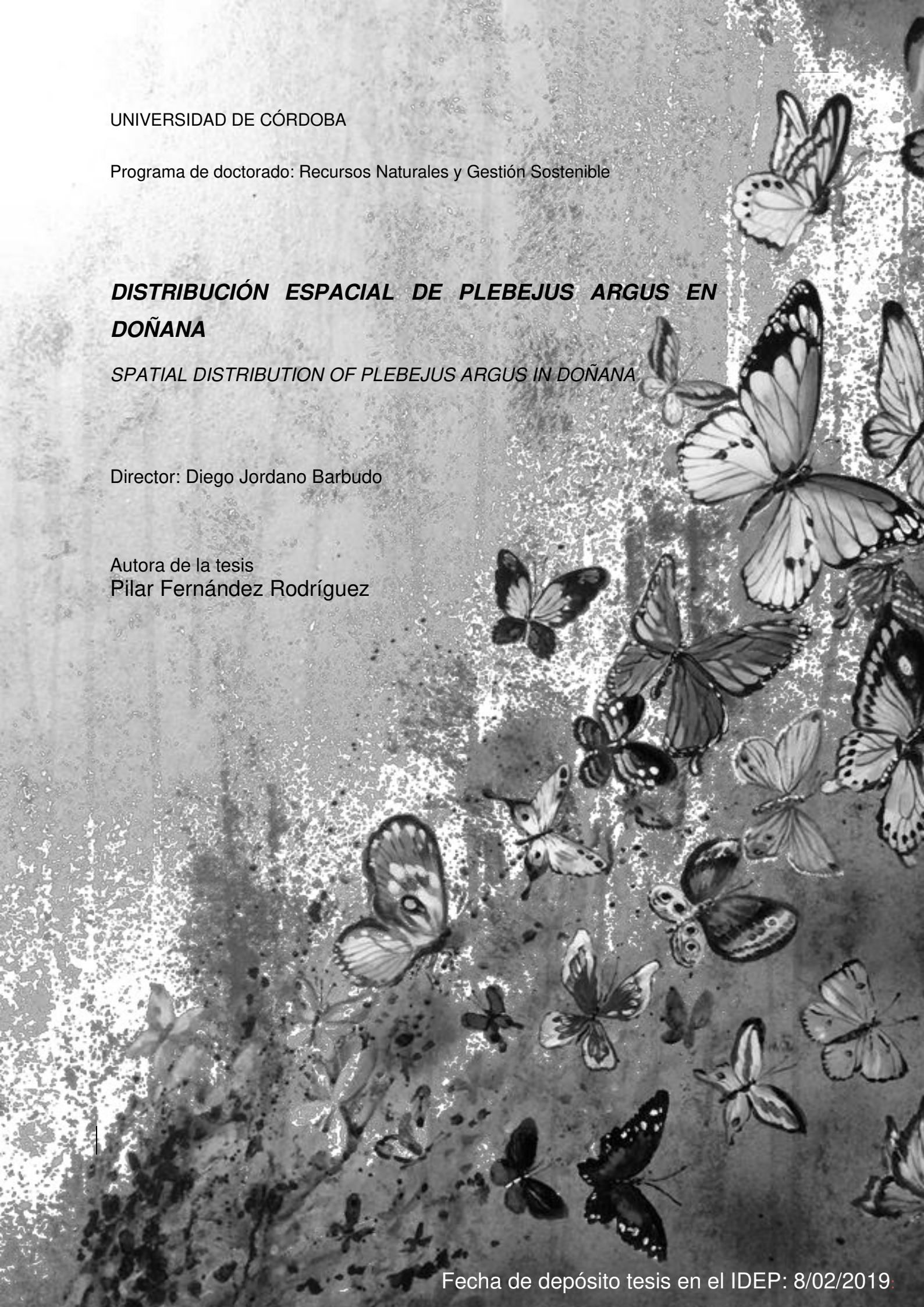

UNIVERSIDAD DE CÓRDOBA

Programa de doctorado: Recursos Naturales y Gestión Sostenible

DISTRIBUCIÓN ESPACIAL DE PLEBEJUS ARGUS EN DOÑANA

SPATIAL DISTRIBUTION OF PLEBEJUS ARGUS IN DOÑANA

Director: Diego Jordano Barbudo

Autora de la tesis
Pilar Fernández Rodríguez

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TITULO: ***DISTRIBUCIÓN ESPACIAL DE PLEBEJUS ARGUS EN DOÑANA***

AUTORA: ***PILAR FERNÁNDEZ RODRÍGUEZ***

Campus de Rabanales

Ctra. Nacional IV, Km. 396 A

14071 Córdoba

https://www.uco.es/ucopress/index.php/es/

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DEPARTAMENTO DE BOTÁNICA, ECOLOGÍA Y FISIOLOGÍA

VEGETAL

UNIVERSIDAD DE CÓRDOBA

TESIS DOCTORAL: DISTRIBUCIÓN ESPACIAL DE *PLEBEJUS ARGUS* EN DOÑANA

Autora: Pilar Fernández Rodríguez

Director: Diego Jordano Barbudo

Córdoba, Marzo de 2019

TÍTULO DE LA TESIS: Distribución espacial de Plebejus argus en Doñana

DOCTORANDO/A: Pilar Fernández Rodríguez

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

Pilar Fernández en su tesis ha profundizado en el análisis de la distribución espacial de mariposas a escala geográfica mediante modelos de distribución de especies, que revelan importante información del rango de valores de distintos factores abióticos que hacen posible su presencia y que puedan mantener poblaciones viables. En particular ha trabajado con la mariposa *Plebejus argus* a diferentes escalas espaciales, investigando además el efecto de factores bióticos como la distribución de la planta nutricia de sus larvas y la de la especie de hormiga con la que mantiene una relación mutualista obligada.

El caso de *P. argus* en Doñana es especialmente interesante: siendo una especie rara en el sur de la Península Ibérica, donde se encuentra formando escasas poblaciones locales de sitios montañosos como Sierra Nevada, en Doñana a pocas decenas de metros sobre el nivel del mar es con diferencia la especie de mariposa diurna más abundante. Aún más sorprendente es que su densidad varía enormemente entre zonas muy cercanas (en apenas 100 metros) sin que se aprecien diferencias en la estructura de la vegetación y del hábitat.

Pilar Fernández ha realizado un enorme trabajo de campo reuniendo un gran volumen de datos que le han permitido determinar la combinación de factores y recursos que hacen posible que *P. argus* sea tan abundante en Doñana y que determinan su peculiar distribución espacial. Esta aproximación se complementa con el trabajo que ha llevado a cabo para analizar los movimientos de las mariposas en relación con la estructura del hábitat. Prueba del gran trabajo realizado por Pilar Fernández son cinco artículos publicados en revistas SCI y 12 comunicaciones en congresos nacionales e internacionales.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 6 de febrero de 2019

Firma del/de los director/es

Fdo.: Dr. Diego Jordano Barbudo

INDICE 1

PLAN DE LA TESIS

La presente Tesis Doctoral está estrechamente relacionada con el desarrollo de dos proyectos de investigación. El primero de ellos, titulado: "*Survival and evolution of species in fragmented landscapes (Fragland)*", Research Network FMRX-CT98-0227 (DG 12-MZLS) del programa Training and Mobility of Researchers (TMR), IV Programa Marco de la Unión Europea, realizado en el Área de Ecología de la UCO en el periodo 1998-2001. En este proyecto de investigación participaron investigadores de siete países europeos actuando como coordinador de todos ellos Ilka Hanski de Finlandia y como coordinador del equipo español Diego Jordano, director de esta Tesis. En este proyecto el objetivo principal del grupo español fue conocer la estructura poblacional de una especie de mariposa, *Plebejus argus*, en un área marginal en el límite sur de su distribución continental: el Parque Nacional de Doñana. Esta era una de las especies diana seleccionadas para los trabajos de investigación a desarrollar por los grupos de investigación participantes en el proyecto "Fragland", del cual hay abundante información disponible en:

http://www.helsinki.fi/science/fragland/index.html

El segundo de ellos, "*Viabilidad y conectividad ecológica de un nuevo modelo de reforestación: aplicación al corredor verde del Guadiamar*" (2007-2010) del que fue Investigador Principal Alejandro Rodriguez, en el que Área de Ecología colaboró estrechamente con la Estación Biológica de Doñana (CSIC). Uno de los principales objetivos de este proyecto fue analizar la respuesta dispersiva de distintas especies en paisajes fragmentados, y concretamente se trabajó investigando los patrones de movimiento de *P. argus* en Doñana, donde esta especie se desenvuelve en un mosaico de hábitats de diferente calidad.

Como puede desprenderse del índice y más adelante de la lectura del manuscrito, se ha optado por una presentación como compendio de artículos ya publicados o en fase de publicación, que es una de las modalidades contempladas por la normativa actual de estudios de Doctorado de la Universidad de Córdoba.

El primer capítulo es una introducción general a este trabajo que trata de describir la importancia del mismo en el contexto actual del conocimiento sobre aspectos de ecología espacial de poblaciones y movimiento de individuos. Se hace además una breve mención a los objetivos globales de la Tesis y se describen también genéricamente algunos de los principales aspectos metodológicos empleados Se incluye, finalmente, una breve descripción del escenario donde se desarrolla el trabajo (Parque Nacional de Doñana) así como de los protagonistas principales: la mariposa, la hormiga mutualista y la planta sobre la que se produce la interacción.

El Bloque I aborda la distribución espacial de *P. argus* a distintas escalas e incluye los capítulos 2 al 5. Estos capítulos se corresponden con cuatro artículos publicados en distintas revistas, que se ordenan de forma coherente para cubrir los objetivos propuestos.

El segundo capítulo trata de la distribución espacial de la especie a escala nacional. Se incluyen en este análisis otras dos especies (*Cyaniris semiargus* y *Pyronia tithonus*) que, como se verá, comparten muchas similitudes en su distribución en la Península Ibérica.

Los siguientes capítulos abordan el análisis de la distribución de la especie a escalas de mucho mayor detalle. El capítulo tercero aborda un modelo de distribución de la especie a escala del Parque Nacional de Doñana mediante sistemas de información geográfica y la influencia de las hormigas mutualistas en esta distribución. El cuarto capítulo analiza la variación espacial de las presencia y abundancia de la mariposa a escala local de 10×10 m en diferentes localizaciones dentro del PND y la influencia que sobre esta distribución tiene la profundidad de la capa freática. Finalmente el último capitulo de este bloque relaciona la abundancia de las mariposas en relación a la arquitectura de las plantas hospedadoras de sus hormigas mutualistas.

En el Bloque II se analizan los movimientos de *P. argus* como un aspecto comportamental estrechamente relacionado con su distribución espacial.

El capítulo 6 propone una metodología original basada en el seguimiento de los movimientos de la especie mediante GPS y su análisis comparativo en distintos hábitats. El capítulo 7 analiza el caso especial del movimiento de *P. argus* a través de los cortafuegos existentes en el Parque Nacional y el papel que pueden jugar estas estructuras como barreras o filtros para el movimiento y dispersión de la especie.

El trabajo incluye finalmente las principales conclusiones que se pueden deducir de las distintas observaciones y experimentos realizados (capítulo 8) y se reseñan finalmente todas las referencias bibliográficas citadas en la introducción (capítulo 1).

RESUMEN DE TESIS

Conservar la biodiversidad existente y comprender los mecanismos que rigen la actual pérdida de especies se ha convertido en una prioridad subyacente en muchos estudios ecológicos. Esta labor se plantea desde varios frentes de actuación y desde distintos niveles. Por un lado, es necesario actuar sobre la especie o especies concretas mediante medidas que mejoren, por ejemplo, la supervivencia (nivel de especie) y, por otro, cada vez es más necesario actuar sobre el hábitat y desarrollar medidas efectivas de conservación (nivel de ecosistemas)

P. argus es un licénido que ha sido estudiado ampliamente en distintos puntos de su área de distribución en Europa y que mantiene relaciones mutualistas con hormigas del género *Lasius, Lasius niger en Doñana*. En Doñana es una especie abundante, fácil de reconocer y que aparece en vuelo de forma explosiva en primavera, lo que la convierte en un candidato ideal como especie indicadora para seguimientos poblacionales.

En este trabajo se trata de conocer la estructura espacial de la población de *P. argus* en el Parque Nacional de Doñana, considerada como población marginal en su área de distribución global y a priori con un grado de fragmentación menor en comparación con otras poblaciones de áreas centrales de su distribución.

Más concretamente, este trabajo de Tesis Doctoral tiene como objetivos globales conocer la estructura espacial, a distintas escalas de detalle (nacional, regional, local) y los movimientos de la mariposa *P. argus* cuando vuelan por hábitats que ofrecen distintos recursos y donde realizan distintas funciones en el Parque Nacional de Doñana. El trabajo se ha estructurado en dos bloques que tratan de responder a estas dos cuestiones básicas: ¿dónde está la mariposa? ¿Cómo y cuánto se mueve?

En el primer bloque de resultados y como una primera aproximación a su distribución espacial, a escala de la Península Ibérica y mediante el uso del software MAXent utilizando como predictores variables bioclimáticas y de usos del suelo, se remarca la singularidad de la población de Doñana mediante una aproximación de modelado sectorizado de la especie en toda la Península.

En capítulos posteriores se analiza la distribución a una escala más local. Para conocer la distribución de la mariposa y los factores causales de la que depende se realizaron prospecciones de mariposas por todo el Parque y medidas descriptivas de la composición y estructura del hábitat y de la presencia de hormigueros de *L. niger*, con las que mantiene una relación mutualista obligada, en cuadrículas a diferentes escalas.

Hemos desarrollado un modelo de distribución de la especie en Doñana a una escala de 1 hectárea, partiendo de la distribución de la hormiga mutualista *L. niger* mediante el empleo de

sistemas de información geográfica. A esta escala local, la utilización de sistemas de información geográfica nos han permitido elaborar un modelo predictivo, espacialmente explícito, basado en la relación entre mariposas y hormigas para una superficie importante del Parque. Este modelo tiene la ventaja de poder predecir la abundancia de mariposas en función de la de su hormiga mutualista, más fácil de medir en muchas épocas del año en las que la mariposa no está presente. Los trabajos de calibración y prueba del modelo han demostrado su validez. La relación positiva significativa de frecuencia predicha de *L. niger* con abundancia y presencia de *P. argus* indican la posibilidad de usar distribuciones modeladas de especies mutualistas en el modelado de sus asociados.

Posteriormente se analiza la distribución espacial de la mariposa a escala de decenas de metros y su relación con la hormiga mutualista, las plantas fuentes de néctar y la distancia a los brezales, como indicador de la humedad del suelo. A esta escala, nuestros datos demuestran que, aunque las distancias entre los puntos más extremos dentro de cada transecto podrían ser fácilmente alcanzadas por adultos de *P. argus*, éstos se concentran en determinadas zonas dentro del transecto, dando lugar a diferencias de abundancia entre parcelas cercanas de hasta dos órdenes de magnitud. Esta variación de abundancia está fuertemente relacionada con la de hormigueros y, en menor medida, con la presencia de plantas fuentes de néctar. Los hormigueros de *L. niger* tienden a situarse en zonas donde la humedad del suelo sea la mayor posible, sin llegar antes al encharcamiento. Tienden, por tanto, a tener su máximo de abundancia en la zona media-baja de las antiguas dunas estabilizadas por el matorral. La relación mariposa-planta hay que contemplarla en un escenario donde la variabilidad ambiental a escala de decenas de metros determinada por la topografía, condiciona la humedad del suelo y la presencia de hormigueros, que son esenciales para explicar la presencia y abundancia de la mariposa.

Finalmente se han realizado varios experimentos para determinar la selección de la planta nutricia de las orugas de la mariposa (*Halimium halimifolium*) y el papel que tiene en esta selección la presencia de la hormiga mutualista *L. niger*. Para ello nos hemos centrado en intentar determinar si existen caracteres morfoestructurales de la misma planta que sean preseleccionados por la hembra grávida o por las hormigas. Para cubrir este objetivo se han tomado distintas medidas de las plantas (altura, diámetros, etc) con y sin hormigueros de *L. niger* y con puestas de *P.argus* o sin ellas en distintos puntos del área de estudio. Las hembras de *P. argus* seleccionan plantas con hormigueros de *L. niger* y, por lo tanto, la morfología de estas plantas se ajusta en una primera instancia a las seleccionadas por las hormigas (mayor altura y diámetro basal, plantas mas leñosas y menos aisladas).

Pero la estructura espacial de *P. argus* no depende sólo de cómo estén estructurados

espacialmente los rodales de hábitat, sino que también resulta básico conocer el grado de conexión entre ellos, que depende a su vez de la capacidad de dispersión individual.

Para ello, en el segundo bloque de resultados, se ha estudiado la capacidad dispersiva de la especie mediante seguimientos de individuos concretos con GPS. El método consiste básicamente en seguir a un individuo recorriendo su misma trayectoria con la función tracking del GPS activada y configurada para que registre posiciones y datos asociados cada segundo (fecha, hora, coordenadas, altitud, velocidad del tramo, distancia recorrida en el tramo).

P. argus no es una mariposa de gran movilidad y capacidad de dispersión. Los experimentos de seguimiento individuales realizados demuestran que la mayoría de individuos se mueven distancias inferiores a 100m., lo que sugiere que encuentran todos los recursos que necesitan (conespecíficos para la reproducción, plantas fuentes de néctar, plantas para la oviposición, etc.) en el mismo área donde han nacido. El patrón de movimiento en *P. argus* parece responder a la heterogeneidad espacial de los recursos fundamentales que las mariposas necesitan para sobrevivir y reproducirse y, por tanto, los atributos básicos del movimiento registrado para esta especie (dirección, velocidad, sinuosidad) cambian sustancialmente con la calidad del hábitat por donde se mueven.

El encuentro entre hábitats de diferente calidad muestra como la mariposa debe tomar decisiones ante el borde. Esta toma de decisiones de cruzar el borde o permanecer en el hábitat actual parece estar fuertemente influenciada por la ganancia neta esperada de la calidad del hábitat.

En este sentido, la extensa red de cortafuegos en Doñana puede estar influenciando la conectividad de las diferentes zonas ya que la red de cortafuegos es mayor en las zonas relativamente más secas, coincidentes con las zonas donde el modelo de hábitat predice menos abundancia de *P. argus* y mayor aislamiento.

Aunque sin problemas aparentes de interconexión dentro de la población de Doñana, globalmente si puede considerarse como una población aislada del resto de las poblaciones peninsulares, que ha perdido la posibilidad de intercambiar individuos con otras poblaciones de su entorno. Aunque en el momento actual es una población de alta densidad relativa, puede tener problemas de conservación derivados de eventuales descensos del acuífero y del manejo de la vegetación (desbroces).

Thesis abstract

Preserving biodiversity and understanding the mechanisms governing the current loss of species has become an underlying priority in many ecological studies. This task is achieved from several fronts of action and from different levels. On the one hand it is necessary to act on the specific species or species through measures that improve, for example, survival (species level), but on the other hand it is increasingly necessary to act on habitats and develop effective conservation measures (ecosystem level).

P. argus is a licaenid widely studied in different parts of its distribution area in Europe that has a mutualistic relationship with ants of the genus *Lasius*, *Lasius niger* in Doñana.

In Doñana it is also an abundant species, easy to recognize and that is on the wing in an explosive manner in spring, which makes it an ideal candidate as an indicator species in population monitoring.

The present work tries to learn the spatial structure of the population of *P. argus* in the Doñana National Park, considered as a marginal population in its global distribution area and *a priori* with a lower degree of fragmentation compared to other populations in core areas of its distribution area.

The main objectives of this thesis were to analyze the spatial distribution at different scales (national, regional, local) and the movements of the species *P. argus* when flying through habitats offering different resources and with different functionality in Doñana national park.

This thesis has been structured in two parts that lead to answering these two basic questions: where is the butterfly? and how and how much does it move?

In the first part of results and as a first approach to its spatial distribution, at Iberian Peninsula scale we used the free software MAXENT, with bioclimatic and land use variables as predictors. The singularity of the population of Doñana is highlighted by a sectorized approach for modeling the species throughout the Peninsula.

Subsequent chapters analyze the distribution on a more local scale. To know the distribution of the butterfly and its causal factors, surveys of butterflies were carried out throughout the Park, together with descriptive measures of the composition and structure of the habitat and the presence of nests of *L. niger* in grids at different scales.

We have developed a species distribution model in Doñana using GIS at a 1 hectare scale, starting from the distribution of the mutualist ant *L. niger*. At the local level, the use of geographic

information systems has allowed us to develop a spatially explicit predictive model based on the relationship between butterflies and ants for an important area of the Park. This model has the advantage of being able to predict the abundance of butterflies based on the presence of their mutualistic ant, which is easier to measure during those periods of the year when the butterfly is not present. The work of calibration and testing of the model have proved their validity. The significant positive relationship of predicted frequency of *L. niger* with abundance and presence of *P. argus* indicates the possibility of using modeled distributions of mutualistic species in the modeling of their associates.

Subsequently, the spatial distribution of the butterfly on a scale of tens of meters is analyzed together with its relationship with the mutualistic ant, nectar source plants and the distance to heathlands, as a subrogated of soil moisture. At this scale, our data show that although distances between the most extreme points within each transect could easily be reached by *P. argus* adults, they are concentrated in certain areas within the transect, resulting in abundance differences between nearby plots of up to two orders of magnitude. This variation of abundance is strongly related to ant nests and, to a lesser extent, to the presence of nectar source plants. Nests of *L. niger* tend to be located in areas where soil moisture is the highest possible, avoiding flooding situations. They tend, therefore, to have their maximum abundance in the low-middle zone of the old scrubland stabilized dunes. The butterfly-plant relationship must be contemplated in a scenario where the environmental variability at the scale of tens of meters, which is determined by the topography, determines the humidity of the soil and the presence of ant nests, which are essential to explain the presence and abundance of the butterfly.

Finally, several experiments have been carried out to determine the selection of the host plant by the caterpillar of the butterfly (*Halimium halimifolium*) and the role played in this selection by the mutualist ant *L. niger*. We have focused on determining if there are any morpho-structural characters of the same plant that are preselected by the gravid female or by the ants. Different measures of the plants (height, diameters, etc.) have been taken with and without nests of *L. niger* and with and without *P. argus* eggs in different points of the study area. The females of *P. argus* in Doñana also select plants with anthills of *L. niger* and, therefore, the morphology of these plants is adjusted in a first instance to those selected by the ants (higher height and basal diameter, woody and less isolated plants).

But the spatial structure of *P. argus* does not depend only on how the habitat fragments are spatially structured, it is also essential to know the degree of connection between them, which in turn depends on the individual dispersal capacity. For this purpose, in a second block of results, the dispersive capacity of the species has been studied tracking individuals with a GPS. This

method basically consists in following an individual going through the same trajectory with the GPS tracking function once activated, configured to register positions and associated data at every second (date, time, coordinates, altitude, speed of the stretch, distance covered in the stretch…) .

P.argus is not a butterfly with high mobility and dispersal capacity. The individual monitoring experiments carried out showed that most individuals can only cover distances below 100m, which suggests that they find all the resources they need (conspecific for reproduction, plants sources of nectar, plants for oviposition ...) in the same area where they were born.

The pattern of movement in *P. argus* seems to respond to the spatial heterogeneity of the fundamental resources that butterflies need to survive and reproduce and, therefore, the basic attributes of the movement recorded for this species (direction, speed, sinuosity) change substantially with the quality of the habitat through which they move.

The confluence between habitats of different quality shows how the butterfly must make decisions at the edge. This decision between crossing the border or either staying in the current habitat may be strongly influenced by the expected net gain of habitat quality.

In this sense, the extensive network of firebreaks in Doñana may be influencing the connectivity of the different areas within Doñana given that the firebreaks network is larger in the relatively drier areas coinciding with the ones where the habitat model predicts less abundance of *P argus* and greater isolation.

Despite this species population shows no interconnection problems within the population of Doñana, it can globally be considered as an isolated one, compared to the rest of the peninsular populations, as it has lost the possibility of exchanging individuals with other populations of its environment. Although at the present time it is a population of high relative density it may have conservation problems derived from possible decreases of the aquifer and vegetation management (clearing).

[CAPÍTULO 1]

INTRODUCCIÓN

CAPÍTULO 1. INTRODUCCIÓN

Conservación de la biodiversidad y hábitats. El paradigma de la fragmentación.

Conservar la biodiversidad existente y comprender los mecanismos que rigen la actual pérdida de especies se ha convertido en una prioridad subyacente en muchos estudios ecológicos. Esta labor se plantea desde varios frentes de actuación y desde distintos niveles. Por un lado es necesario actuar sobre la especie o especies concretas mediante medidas que mejoren, por ejemplo, la supervivencia (nivel de especie), y, por otro, es cada vez más necesario actuar sobre el hábitat y desarrollar medidas efectivas de conservación (nivel de ecosistemas) (Brooks et al. 2002; Zimmerer 2006). En los últimos tiempos el foco de atención está centrado en cómo, en un mundo sujeto a cambios drásticos, la pérdida de las interacciones entre especies es un paso previo a la desaparición de las especies en sí mismas (Valiente-Bauet et al. 2015).

A pesar del gran esfuerzo empleado, los intentos por conservar las especies a gran escala han resultado a menudo infructuosos (Adams and Carwardine 1994; Delibes 2001). El ritmo actual de extinciones es incluso superior al de las grandes extinciones que conocemos, tres a cuatro veces por encima de los ritmos de extinción base que se consideran normales (Gastón et al. 2000; Brooks et al. 2002; Barnosky et al. 2011).

La pérdida de biodiversidad tiene un origen muy variado, pero en una gran mayoría de casos está relacionada en último término con la intervención del hombre sobre la naturaleza. A la acción directa de éste sobre una especie concreta (extinciones debidas a la caza excesiva, sobrepesca, etc.) hay que sumar el efecto de las especies invasoras (Gurevitch and Padilla 2004), el deterioro y/o pérdida de hábitat debido a la deforestación, a la transformación de hábitats naturales para usos agrícolas y/o ganaderos (León-Cortez et al. 2000; Luoto et al. 2001; Gurevitch and Padilla 2004), urbanización (León-Cortez et al. 2000; Gallou et al. 2017; Melliger et al. 2018), o a la contaminación. Estos procesos afectan de forma generalizada a muchos territorios, llevando a una variación drástica en la cantidad y/o calidad de los recursos y condiciones para las especies. En los últimos tiempos, el cambio climático se presenta como la mayor amenaza para la biodiversidad (Gallou et al. 2017) lo que unido al aumento en la fragmentación de los hábitats impide y dificulta el mantenimiento de las especies que van quedando aisladas e incapaces de colonizar nuevos hábitats. El paisaje, que antes podía considerarse como un continuo sin barreras para las especies (sujeto el hábitat a condicionantes abióticos y bióticos), se caracteriza hoy día por su creciente fragmentación, que afecta tanto a paisajes humanizados como a áreas consideradas puntos calientes de biodiversidad (Brooks et al. 2002; Vidal y Rendón-Salinas 2014).

Básicamente, la fragmentación se refiere a la subdivisión de una porción del hábitat de una especie en fragmentos de tamaño cada vez menor, incrementándose el aislamiento entre fragmentos a medida que avanza la destrucción del hábitat (Andrén 1994; Bascompte and Solé 1996; Hanski 1999).

Estos fragmentos tienden además a mantener menos especies especialistas de hábitat y sufren, por el contrario, un incremento en las especies generalistas, además de alteraciones en las interacciones ecológicas (Harrison and Bruna 1999; Valiente-Bauet et al. 2015). El grado de fragmentación del hábitat es tal, que se hace necesario un conocimiento exhaustivo de los posibles efectos sobre la dinámica poblacional de las especies afectadas (Thomas and Hanski 1997; Lane et al. 2001). Para entender la actual línea de pensamiento teórico resulta útil perfilar cómo ha evolucionado el conocimiento sobre el funcionamiento de las poblaciones.

Ecología espacial y metapoblaciones.

Tradicionalmente se ha considerado que los individuos forman parte de poblaciones cuya dinámica (natalidad, mortalidad, emigración e inmigración) va a estar regida en último término por la densidad (competencia intraespecífica) y por la interacción con otras especies (competencia interespecífica) (Begon et al. 1987). Sin embargo, pronto resultó obvio que el hábitat juega un papel fundamental en la dinámica de las poblaciones.

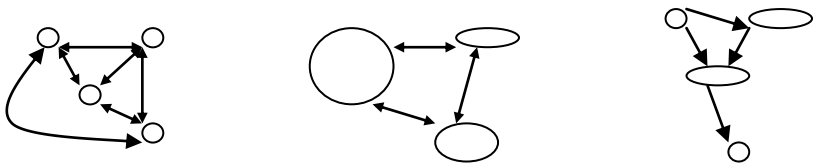
Ya en 1954, Andreawartha y Birch introdujeron la idea de que una población natural que ocupe un área geográfica extensa frecuentemente está constituida por diferentes poblaciones locales o colonias. Estos autores resaltaban la idea de que la demografía de distintas poblaciones locales o colonias puede a menudo mostrar diferentes tendencias en un tiempo dado.

Sabemos ahora que la estructura espacial de las interacciones ecológicas afecta a las poblaciones tanto como los índices de natalidad y mortalidad, la competencia y la depredación.

Un primer paso en la comprensión del papel que el espacio juega en la biodiversidad fue la teoría de biogeografía de islas de McArthur y Wilson (1967) que interpretaba el patrón espacial de una comunidad, y que tiene su equivalente a nivel de poblaciones en la teoría de metapoblación de Levins 1970. Se trata de una población formada por un conjunto de poblaciones locales que ocupan fragmentos de hábitat adecuado inmersos en una matriz de hábitat inhóspito y que pueden extinguirse localmente o ser recolonizadas desde otros fragmentos ocupados. Un fragmento de hábitat adecuado es, por tanto, un área continua que tiene todos los recursos necesarios para la persistencia de una población local.

La dinámica de la metapoblación está gobernada por el balance entre colonización (ocupación de fragmentos de hábitat vacantes) y extinción (extinción de poblaciones locales), lo que permite la

supervivencia de la especie a una escala regional a pesar de la extinción de poblaciones locales (Hanski et al. 2017). La línea de pensamiento de Levins es conceptualmente similar a la seguida por MacArthur y Wilson, pero con la diferencia esencial de que ahora son las poblaciones de una única especie las que están sujetas al ritmo de colonización-extinción.



a) b) c)

Figura 1 Esquema de las diferentes disposiciones de fragmentos de hábitat y grado de conexión según distintos modelos. a) modelo de metapoblación de Levins, con fragmentos equivalentes en área y forma y conexión completa, b) fragmentos de distinta magnitud y todos conectados entre sí, c) fragmentos de distinta magnitud y diferente grado de conexión

El grado de conexión entre los fragmentos de hábitat ocupados por la especie, el tamaño de los fragmentos y la existencia de hábitat adecuado son los tres ejes fundamentales en la dinámica de metapoblaciones (Lewis et al. 1997).

El papel fundamental de la dispersión ha llevado a pensar en la existencia de corredores entre fragmentos que faciliten el movimiento de los individuos o, por el contrario, de barreras que lo dificulten. Aunque hay controversia sobre la eficacia de los corredores para facilitar la conexión entre fragmentos y para mitigar las consecuencias de la pérdida de hábitats en la disminución de biodiversidad (Beier and Reed, 1998; Harrison and Bruna, 1999), numerosos estudios remarcan su importancia (Gilbert-Nortin et al. 2010; Christie and Knowles 2015). Los conocidos como hábitats *"stepping stone"* (algo así como las piedras que usamos para saltar de una orilla a otra en el río) son también elementos que incrementan las posibilidades de dispersión de las especies (Lewis et al. 1997). Existen además evidencias de que la configuración espacial es importante, principalmente por efectos de borde (Harrison and Bruna 1999; Schultz et al. 2012). Por otro lado, la matriz, que en los modelos más simplificados se considera homogénea y sin valor como hábitat, gana cada vez más importancia y se considera heterogénea en su respuesta de permeabilidad al movimiento de los individuos, variando la conectividad entre fragmentos (Revilla and Wiegand 2008). Podría decirse que se ha pasado de considerar un paisaje binario de hábitat y no hábitat a un paisaje en una escala de grises en el que la calidad del hábitat y su heterogeneidad adquieren un papel cada vez más importante. Todos estos factores se incorporan en un modelo más real, la

ecología del paisaje, que se ocupa de describir la compleja estructura física del ambiente y de los movimientos de individuos y recursos en él (Luoto et al. 2001; Morales et al. 2010).

En esta simplificación de la dinámica espacial aún quedan muchos interrogantes. Pese a todo, estas teorías espaciales han llevado a considerar estrategias de conservación como la creación de corredores para promover la dispersión o diferentes configuraciones del hábitat remanente que puedan servir para aminorar los efectos de la pérdida y fragmentación del hábitat (Harrison and Bruna 2001; Pereira and Rodriguez 2010; Morales et al. 2010). Tal es el caso, por ejemplo, de la estrategia de conservación que supuso la creación del Corredor Verde del Guadiamar, que trata de favorecer la conectividad entre los ecosistemas de Sierra Morena y los del entorno de Doñana (Montes, Arenas y Borja 2003).

Distribución espacial de especies.

Para establecer la estructura espacial de una población es necesario definir por tanto el hábitat adecuado y conocer el grado de conexión entre los fragmentos de hábitat, así como la capacidad colonizadora de la especie. Por un lado es preciso conocer la respuesta de la especie a distintas condiciones físico -químicas, qué recursos está utilizando y grado de disponibilidad de los mismos, cómo responde a predadores y competidores, respuesta a variables ambientales, y cómo estos influyen en su tasa de natalidad, de mortalidad y de migración de la población (Begon et al. 1987) para así valorar en lo posible qué entiende la especie por hábitat adecuado. Por otro, necesitamos conocer la capacidad de dispersión de la especie y su capacidad colonizadora, y como consecuencia de todo, la variabilidad genética de las poblaciones, que nos habla del grado real de separación y aislamiento de las poblaciones (Zhan et al. 2014; Hanski et al. 2017).

Pero la estructura espacial de una especie no suele ser fácil de definir. La especie no siempre responde a factores apreciables a nuestra vista y pueden pasarnos desapercibidos elementos que son, en realidad, los determinantes de la distribución de la especie. Por ejemplo, la distribución de una especie puede estar condicionada por un factor operando a una escala de tiempo superior a la de nuestro trabajo (por ejemplo, periodos de sequía cíclicos que condicionen la expansión o retroceso de una especie) o puede ser respuesta a una acción sobre la especie a varios cientos o miles de km. del punto de trabajo. Así, por ejemplo, durante la invernada de 2014 las mariposas monarca (*Danaus plexippus*) disminuyeron espectacularmente el área que ocupan en los bosques de pino oyamel de México, lo que afectará las mariposas que migren hacia Estados Unidos y Canadá en la primavera siguiente y viceversa (Vidal y Rendón-Salinas 2014). Conocer la escala temporal y espacial a la que un organismo percibe y responde al medio es pues una materia fundamental en ecología (Wiens 1989).

Estrategias de análisis en los estudios de distribución de especies

Los estudios de biodiversidad han llevado, por un lado, al desarrollo, de estrategias globales para entender la distribución de las especies a escalas amplias, nacionales o globales y por otro lado, a estrategias más locales en hábitats concretos, en las que se elaboran los estudios necesarios que analicen la distribución local observada de una especie. En el primer caso, se trata de utilizar algoritmos para predecir la distribución potencial de una especie en un área geográfica determinada en base a datos de ocurrencia y a datos ambientales, principalmente climáticos. Esta técnica se conoce como modelización de la distribución de especies o modelización de nicho. En el segundo caso, se trata de diseñar protocolos de trabajo a escalas locales en los que se investiguen los requerimientos de hábitat de la especie, el grado de fragmentación de sus poblaciones, la capacidad de movimiento y las principales amenazas.

Para facilitar el trabajo en este aspecto, desde hace años se están empleando herramientas de análisis espacial, los Sistemas de Información Geográfica (SIG), que permiten generar mapas de calidad de hábitat de cualquier especie a partir de modelos matemáticos de hábitat. Una vez se conoce la respuesta de la especie al hábitat mediante la modelización de un reducido número de variables, el modelo de hábitat predice la distribución de la especie en toda la región de estudio. Hay numerosos estudios que utilizan SIG en la elaboración de modelos. Se ha trabajado con muchas especies de vertebrados (Mladenoff et al. 1999; Gurnell et al. 2002; Niedziałkowska et al. 2006), e invertebrados (Fleishman et al. 2001; Graze and Maremootoo 2002; Luoto et al. 2002), tanto a nivel de especie (Glentz et al. 2001 Fleishman et al. 2001; Osborne et al. 2001; Graze and Maremoto 2002; Gurnell et al. 2002) como de comunidades (Guisan and Zimmermann, 2000).

Las técnicas de **modelado ecológico** buscan una relación estadística o matemática entre los datos disponibles sobre la distribución de una especie y diferentes variables que describen las condiciones ambientales, para posteriormente aplicar esta relación al resto del área de estudio.

Los llamados modelos de distribución de especies (MDE o SDM en inglés) han experimentado un gran auge en los últimos años a expensas del desarrollo de nuevas y accesibles técnicas estadísticas y al desarrollo de los sistemas de información geográfica (SIG) (Guisan and Zimmermann 2000). Los MDE son una herramienta en pleno desarrollo y expansión; continuamente surgen nuevos métodos, estrategias y aproximaciones tanto a nivel de especie (Elith et al. 2010), como a nivel de comunidad (Rahbek et al. 2007; Maguire et al. 2015). Se han desarrollado numerosas herramientas de análisis y modelización de uso relativamente sencillo (MaxEnt, Divagis, etc) (Phillips et al. 2006) que facilitan el desarrollo de modelos no subjetivos

para predecir lo adecuada que es un área para el desarrollo de una determinada especie en relación con las condiciones ambientales (p. ej. clima, suelo, topografía, etc.).

Los datos necesarios en el modelado suelen proceder de colecciones, atlas y publicaciones y suelen ser datos de solo presencia (Elith et al 2010). También existen modelos con datos de presencia y ausencia de las especies, aunque normalmente es más difícil disponer de datos de ausencias (Manel et al. 2001; Elith et al. 2010), y modelos relacionados con datos de abundancia (Cawsey et al. 2002; Hill et al. 2017).

A la hora de seleccionar las variables predictoras hay que tener en cuenta tanto la escala espacial como la resolución del análisis (Guisan and Zimmermann 2000; Luoto et al. 2007). Como variables predictoras normalmente se utilizan aquellas que operan en la escala de trabajo considerada (Figura 2, Pearson and Dawson 2003).

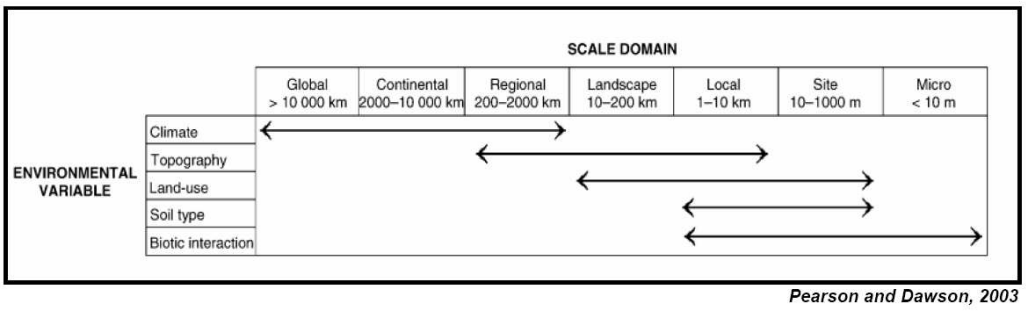


Figura 2. Variables ambientales empleadas en los modelos de distribución de especie según la escala de trabajo. Modificada de Pearson and Dawson 2003.

Numerosas de estas variables predictoras están disponibles de forma gratuita en Internet, como es el caso de variables climáticas disponibles a escala global en WorldClim, tanto para el periodo actual como para un horizonte temporal futuro según varios escenarios de cambio climático (Hijmans et al. 2005). Así mismo, están disponibles variables como usos del suelo (corine) (EEA 1995), pH del suelo, insolación, altitud (WorldClim Hijmans et al. 2005), etc. La modelización de la distribución potencial de una o varias especies utilizando datos ambientales de escenarios futuros previstos por modelos de cambio climático, permiten obtener predicciones de cómo afectará el cambio climático a las especies (p. ej. Romo et al. 2015; Obregón et al. 2016; Zarzo-Arias et al. 2019).

Conexión entre fragmentos de hábitat. Análisis del movimiento.

Además de definir el hábitat adecuado es necesario conocer el grado de conexión entre los fragmentos de hábitat y la movilidad de los individuos. La dispersión, desde una perspectiva espacial, es el nexo de unión entre las poblaciones locales dentro de la red de hábitat adecuados (Hanski 1998). La capacidad de las especies de sobrevivir a largo plazo en hábitats fragmentados depende de su habilidad para dispersarse, permitiendo el flujo genético y la (re)colonización de los fragmentos de hábitat (Harrison et al. 1988; Clobert et al. 2004; Pierce et al. 2014; Hanski et al. 2017). El análisis del movimiento es crucial para la conservación de especies en paisajes fragmentados. El comportamiento de movimiento específico en cada hábitat es clave para entender cómo conectar las poblaciones, y la demografía específica de cada hábitat es fundamental para la gestión de los hábitats (Schultz et al. 2019).

La capacidad de movimiento estará condicionada, entre otras cosas, por cómo percibe la especie el hábitat en el que se mueve (continuo *vs.* fragmentado) y por cómo lo 'utiliza' (recursos coincidentes o separados). Además, la percepción que nosotros tengamos de su hábitat va a depender en gran medida de esta capacidad de dispersión. Cuanto más sedentaria sea la especie más se adecuará el lugar donde la encontremos con sus preferencias en hábitat, mientras que, por el contrario, especies migradoras aprovecharán recursos muy variados e incluso separados en el espacio y/o tiempo y resultará más difícil definir los condicionantes de hábitat. Es decir, para un nivel de resolución determinado, en una especie con una gran capacidad de dispersión esperaríamos que la correspondencia entre la abundancia y la calidad del hábitat fuera menor que en una especie mucho más sedentaria. Para especies especialistas en el uso de hábitats se han utilizado con éxito modelos de movimiento espacialmente explícitos para predecir la expansión de una población establecida por traslocación de individuos, siendo dichos modelos, por tanto, herramientas potenciales para seleccionar áreas de liberación óptimas y con ello mejorar la planificación y los resultados de futuras traslocaciones (Rytteri et al. 2017).

Además, dado que cada movimiento puede deberse a muy diferentes propósitos y puede modificar tanto las condiciones locales del fragmento de partida como del de llegada, no es tan fácil y obvio predecir su impacto global en la dinámica ecológica y evolutiva de la metapoblación (Clobert et al. 2004). Por otro lado, es posible encontrar en la naturaleza agrupaciones locales de individuos en sistemas con individuos muy móviles, con algún tipo de respuesta de agregación en fragmentos favorables (Hassel and May 1974) pero sin estructura de metapoblación. Un ejemplo podrían ser la concentración de licénidos en 'bebederos' o los dormideros de las mariposas de la familia Danaidae. Encontrarlos allí no es prueba de que formen estructura de metapoblación.

Metodologías empleadas para describir el movimiento

Numerosas metodologías se han empleado para el análisis del movimiento de los individuos. Entre todas destaca la técnica del marcado y recaptura (Lewis et al. 1997; Munguira et al. 1997; Brommer and Fred 1999; Baguette, 2003; Gutiérrez et al 2004; Nowicki and Vrabec 2011; Habel et al. 2018) que aporta, por ejemplo, estimas de las distancias euclídeas alcanzadas por los individuos, pero no aporta información sobre el movimiento en sí, ni sobre la respuesta del individuo en hábitats concretos o ante estructuras de diferente grado de permeabilidad al paso. Una limitación de la técnica es que raramente muestrea individuos con la misma probabilidad de recaptura, llevando a una subestima de los índices de movimiento cuando los individuos menos móviles son recapturados con una frecuencia desproporcionadamente mayor, escapando al observador los desplazamientos más largos (Turchin et al. 1991).

Un paso más en los estudios del movimiento es el empleo de técnicas que permitan el seguimiento de individuos concretos de una forma continua. La trayectoria de un individuo a través del espacio y tiempo puede ser asemejada a una serie de desplazamientos lineales, de los que puede registrarse velocidad, dirección, distancia y tiempo entre desplazamientos. En el caso de insectos, se han hecho por ejemplo estudios siguiendo los individuos visualmente (Conradt et al. 2000; Dover and Fry 2001: Ries and Debinski 2001)) y mediante el uso de banderas señalizando los puntos de parada o de giros (Turchin et al. 1991), tarea complicada cuando las distancias son superiores a 200 m. Los recientes avances en el uso de radar armónico (Riley et al 1996; Milanesio et al. 2017) han mejorado el estudio de los movimientos debido a la posibilidad de extender el rango de detección mucho más allá de la visión humana. El radar armónico se ha utilizado por ejemplo para realizar un seguimiento individual de abejas y abejorros a varios cientos de metros (Osborne et al. 1999; Capaldi et al. 2000; Milanesio et al. 2017) o mariposas (Cant et al. 2005; Ovaskainen et al. 2008). El peso del dispositivo es, sin embargo, una limitación de la metodología, y, aunque en *Inachis io y Aglais urticae* el peso del transponder representaba alrededor del 4% y el 8% de su peso corporal, respectivamente (Cant et al. 2005), para los insectos más pequeños el uso de este tipo de "transponder" es irrealizable debido tanto al peso corporal como a la dificultad de manipulación.

En nuestro caso utilizaremos una técnica novedosa para tratar de seguir con la mayor precisión posible las trayectorias concretas de distintos individuos de *P. argus,* utilizando la función track de receptores GPS. Estas trayectorias nos han permitido conocer con gran detalle parámetros de los movimientos de estos individuos tales como la velocidad, dirección, ángulos de giro, sinuosidad, etc. cuando se desplazan en distintos hábitats.

¿Qué mecanismos explican el movimiento?

La capacidad de movimiento es fundamental para la vida y el desarrollo de la mayoría de las especies animales. Puede estar motivada por una disminución de algún recurso por debajo de un límite marcado, por evitar conflictos por el uso de determinados recursos (alimentación, refugio, cópula) (Fronhofer et al. 2015). Cuando la dispersión es motivada por una búsqueda de refugio, alimentación o territorio el proceso se considera dependiente de la densidad (Fred et al. 2006; Nowicki and Vrabec 2011; Astudillo et al. 2012; Kalarus et al. 2013; Fronhofer et al. 2015; Baigas et al. 2017). Por el contrario cuando la causa es la búsqueda de reproducción con individuos no emparentados es un proceso independiente del número de individuos (Hansson, 1991). En este sentido es en el que se define el concepto de *distribución ideal libre* (Clobert et al. 2004), que sostiene que un individuo cambiará de hábitat solo cuando obtenga más beneficio al moverse que al quedarse en el lugar donde esté.

El enfoque clásico normalmente no considera el proceso de movimiento en sí, sino únicamente su consecuencia final, es decir, la traslocación de individuos de un lugar a otro. En estos estudios, la dispersión se va complicando desde procesos de difusión (Okubo 1980) hasta procesos basados en lo que se conoce como paseo al azar o "random walk" (Bartomeus et al. 2005). Sin embargo, posteriormente se ha comprobado que los patrones de dispersión de los individuos pueden ser más complejos que un simple paseo al azar, respondiendo a diversos factores ambientales y, entre otros, la calidad del hábitat y la naturaleza del borde de estos hábitats, (Revilla et al. 2004; Conradt and Roper 2006, Barton et al. 2009; Baigas et al. 2017). Ciertos detalles del comportamiento individual e interacciones que, por ejemplo, concentren los individuos en algunas localizaciones, se oponen al movimiento por simple difusión de los individuos (Peèr et al. 2004; Zhan et al. 2014; Fronhofer et al. 2015).

Surgen, por tanto, toda una serie de modelos de movimiento de dispersión animal que asumen que el proceso de movimiento puede ser descrito como "random walk" o mediante versiones modificadas del mismo (Bartumeus et al. 2005; Barton et al. 2009). Se basan en la suposición de que el movimiento puede ser descrito por los atributos estadísticos de la longitud del paso y las distribuciones del ángulo de giro. Las decisiones del movimiento se suponen aleatorias.

Sin embargo, en gran parte de los movimientos de animales la dirección es persistente. El animal cambia su dirección gradualmente paso a paso. Este es el modelo comúnmente conocido como paseo aleatorio correlacionado (correlated random walk, CRW) (Kareiva and Shigesada 1983; Barton et al. 2009). En estos CRW, la dirección elegida en el paso siguiente está relacionada con la dirección del paso actual. Lo que es elegido al azar es el cambio en la dirección o ángulo de giro entre ambos pasos.

En la naturaleza los recursos están a menudo espacialmente autocorrelacionados (Conradt et al. 2003), es decir, las plantas que por ejemplo sirven de alimento tienen una distribución en parches y están rodeadas con frecuencia por otras plantas alimenticias. En estos casos un encuentro con recursos revela información sobre la probabilidad de encontrar recursos adicionales cerca. Surgen así modelos basados en el concepto de la zona de búsqueda restringida (ARS, Kareiva y Odell 1987, Benhamou 2007; Pinaud 2008) que asumen que los individuos recuerdan pasados encuentros y disminuyen como consecuencia la rectitud (correlación) de su "random walk" al detectar el recurso. La búsqueda es más intensa en áreas de alta densidad de recursos. Si equiparamos "agregaciones de recursos" con el término "fragmentos de hábitat" los modelos ARS generan movimiento más o menos rectos, que puede ser calificados como de dispersión (Ronce 2007; Baigas et al. 2017) en los tramos inter-parches, que pueden ser diferenciados del comportamiento de búsqueda de recursos dentro del fragmento de hábitat, mucho más sinuosos.

En los últimos años se han desarrollado una serie de enfoques en los que se modela la decisión del movimiento basada en información 'aprendida' individualmente o incluso evolutivamente sobre la distribución espacial de, por ejemplo, recursos alimenticios (Wolf et al. 2009; Morales et al. 2010; Schultheiss et al. 2013; Fagan et al. 2013).

Papel de las mariposas en estudios espaciales. El caso de *Plebejus argus*.

Se han desarrollado variados estudios de dinámica poblacional y ecología espacial, con especies de mariposas entre los que destacan, por citar algunas, *Brenthis ino* (Weyer and Schmitt 2013), *Phengaris alcon* (Wynhoff et al. 2015; *Cupido minimus, Melanargia galatea y Aporia crataegi* (Baguette et al. 2000), *Hesperia comma* (Thomas and Jones 1993), *Melitaea cinxia* (Kuussaari et al. 1996), *Parnassius mnemosyne* (Luoto et al. 2001), *Lycaena phlaeas* (León Cortez et al. 2000), *Lycaeides melissa samuelis (*Guiney et al*. 2010), Satyrium ilicis (Maes et al. 2014) Wheeleria spilodactylus* (Menéndez, and Thomas 2000), (*Zerynthia polyxena,* Örvössy et al. 2014), *Parnassius apollo* (Fred et al. 2006).

La mayoría de los lepidópteros requieren ciertas plantas alimenticias durante su etapa larval y plantas productoras de néctar, estructuras de apareamiento y reposo durante su etapa adulta (Dennis et al. 2003; Liu et al. 2006). A su vez, en la etapa adulta, los machos y hembras pueden tener diferentes requerimientos de hábitat debido a su diferente comportamiento. En el caso de los lepidópteros, los machos invertirán tiempo considerable en encontrar sitios apropiados para maximizar su éxito de apareamiento, mientras que las hembras invertirán tiempo en encontrar los lugares más apropiados para la puesta de huevos (Scott 1975). Por lo tanto, la calidad del

hábitat de las diferentes etapas y los adultos ya sean machos o hembras podría estar asociada a diferentes conjuntos de variables ambientales y consecuentemente el hábitat de una especie dada se definirá por la unión de estos recursos y condiciones (Anthes et al. 2003; Fred et al. 2006; Dennis et al. 2006, Vanreusel and Van Dyck 2007).

La variación espacial y temporal de los recursos tiene un papel fundamental en la microdistribución de las mariposas en el campo (Wiklund and Ahrberg 1978; Stefanescu and Traveset 2009). Por ejemplo, la disposición espacial de los recursos larvales (plantas alimenticias) y de los recursos adultos (fuentes de néctar) limita el movimiento de las mariposas (Brommer and Fred 1999; Anthes et al. 2003; Zhan et al. 2014). Si los patrones espaciales de estas variables no coinciden, esto podría tener consecuencias para la ocurrencia de la especie, ya que la dinámica de la población dependerá de la dispersión exitosa entre los sitios separados que contienen los recursos y condiciones para las diferentes etapas y sexos (por ejemplo, Fred et al. 2006) y en las opciones de comportamiento (Auckland et al. 2004).

OBJETIVOS GENERALES

La presente tesis tiene como objetivo global conocer la estructura espacial y los movimientos de *P. argus* en el área del Parque Nacional de Doñana. *Plebejus argus* es un licénido que ha sido estudiado ampliamente en distintos puntos de su área de distribución en Europa. Diferentes metapoblaciones de *P.argus* muestran tantas variaciones en su estructura espacial como estructuras de metapoblaciones descritas existen en todas las especies estudiadas hasta ahora. La diferencia en velocidad y en el patrón de extinciones y colonizaciones locales en diferentes metapoblaciones de *P.argus* es aparentemente debida a diferencias en la distribución del tamaño de los fragmentos y de la dinámica de vegetación, no a diferencias en la mariposa en sí. De forma general se ha visto que hábitats tipo "Stepping stone" son cruciales en la creación y mantenimiento de un sistema de metapoblaciones de la mariposa *P.argus* (Thomas and Harrison, 1992). El presente trabajo surge de la importancia que puede tener conocer la estructura espacial de *P.argus* en el Parque Nacional de Doñana, considerada como población marginal en su área de distribución global y *a priori* con un grado de fragmentación menor en comparación con otras poblaciones de la mariposa en distintas áreas marginales y centrales de su área de distribución. Se trata, además, de una especie abundante, fácil de reconocer y que aparece en vuelo de forma explosiva en primavera, lo que la convierte en un candidato ideal como especie indicadora en seguimientos poblacionales dentro de su área.

Una vez establecida su área de distribución y los condicionantes de hábitat será posible identificar las posibles alteraciones debidas a manejos actuales y futuros de la zona y poder reaccionar en consecuencia para implementar medidas para su conservación.

Para ello nos planteamos una serie de objetivos parciales que tratan de profundizar en el conocimiento previo que se tiene de esta especie en Doñana, en aspectos relacionados con su dependencia de factores físicos e interacciones con otras especies y entre ellos:

Se pretende analizar por un lado la distribución espacial a distintas escalas (nacional, regional, local) y, por otro, conocer atributos básicos del movimiento de la especie (dirección, velocidad, ángulos de giro) cuando vuelan por hábitats de distintas calidades, que ofrecen distintos recursos y donde realizan distintas funciones, así como cuando alcanzan el borde entre dos hábitats de diferente calidad (diferente grado de favorabilidad).

MATERIAL Y MÉTODOS

APROXIMACIÓN METODOLÓGICA Y RESULTADOS DERIVADOS DE LA PRESENTE TESIS

En el presente trabajo se desarrollan una serie de experimentos independientes cuyos resultados nos permitirán acercarnos más al conocimiento sobre la dinámica y estructura poblacional de *P.argus* en España y más específicamente en Doñana. Cada capítulo de resultados se corresponde con un artículo ya publicado (5) o en revisión (1) en la actualidad.

En un primer capítulo de resultados (capitulo 2) hemos desarrollado un modelo de distribución potencial de *Plebejus argus* en la Península Ibérica, mediante el uso del software MAXent, de libre distribución, y utilizando como predictores variables bioclimáticas y de usos del suelo, y donde se remarca la singularidad de la población de Doñana mediante una aproximación de modelado sectorizado de la especie en toda la Península. Se presenta aquí la publicación en *Ecological modelling, (2015)* con el título "Living on the edge in species distribution models: the unexpected presence of three species of butterflies in a protected area in southern Europe"

El resto de capítulos muestran una serie de experimentos centrados ya en el Parque Nacional de Doñana excluyendo las áreas de marisma y aquellas áreas de acceso prohibido por nidificación de especies protegidas.

Para conocer la distribución de la mariposa y los factores causales de la que depende se realizaron prospecciones de mariposas por todo el Parque y medidas descriptivas de la composición y estructura del hábitat y de la presencia de hormigueros de *L. niger,* con las que mantiene una relación mutualista obligada, en cuadrículas a diferentes escalas. Finalmente, basándonos en la información obtenida se ha elaborado un modelo de hábitat mediante sistemas de información geográfica.

En el capítulo 3 desarrollamos el modelo de distribución de la especie en Doñana partiendo de la distribución de la hormiga mutualista *Lasius niger*. Los resultados se han publicado en *Ecological Aplications (2005),* con el título "Habitat distribution models: are mutualist distributions good predictors of their associates?".

Los resultados a escala local se presentan en el capítulo 4 publicado en *Journal of Insect Conservation (2017)* con el título "Water availability drives habitat quality for the butterfly *Plebejus argus* in a Mediterranean sand dune landscape".

Por otra parte, se han realizado varios experimentos para determinar la selección de la planta nutricia y el papel que tiene en esta selección la presencia de la hormiga mutualista *L. niger* (capitulo 5). En la selección de plantas nutricias nos hemos centrado en intentar determinar si existen caracteres morfoestructurales de la misma planta que sean preseleccionados por la hembra grávida o por las hormigas. Para ello se han tomado distintas medidas de las plantas (altura, diámetros, etc) con y sin

hormigueros de *L. niger* y con puestas de *P. argus* o sin ellas, en distintos puntos del área de estudio. Los resultados se han publicado en *Shilap revista de lepidopterología, (2016*) con el título "Host plant selection in *Plebejus argus* (Linnaeus, 1758) and its mutualistic ant. The role of plant architecture".

La estructura espacial de *P. argus* no depende sólo de cómo estén estructurados espacialmente los rodales de hábitat, sino que también resulta básico conocer el grado de conexión entre ellos, que depende a su vez de la capacidad de dispersión individual. Con este fin se ha estudiado la capacidad dispersiva de la especie mediante seguimientos de individuos con GPS. El método consiste básicamente en seguir a un individuo determinado a lo largo de su misma trayectoria exacta con la función tracking del GPS activada configurada para que registre posiciones y datos asociados cada segundo (fecha, hora, coordenadas, altitud, velocidad del tramo, distancia recorrida en el tramo). Con la tabla de datos se crea en ArcGIS una capa de tipo polilínea que permite visualizar la trayectoria seguida por la mariposa y analizarla en relación con otras capas de información relativa a la estructura del hábitat. De esta forma se tiene un dibujo muy exacto del movimiento del individuo, con tiempo, velocidad, ángulos de giro y distancias recorridas en cada segundo de seguimiento. Este objetivo se aborda en los capítulos 6 (artículo publicado en *Journal of Insect Behaviour, 2016*, con el título "Using GPS technology to analyze the movements of *Plebejus argus* in a patchy landscape") y 7 donde se analiza específicamente cual es la respuesta de movimiento de los individuos cuando llegan al borde de un fragmento de hábitat adecuado y se encuentran con un ambiente hostil que podría actuar como barrera a la dispersión, como es un cortafuegos. Este trabajo constituye un artículo actualmente en revisión en la revista Animal Conservation.

ÁREA DE ESTUDIO.

El Parque Nacional de Doñana (PND) se sitúa en la costa suroeste de la Península Ibérica, en las provincias de Huelva y Sevilla, en la margen derecha del tramo final del río Guadalquivir. Comprende una extensión de 50.720 Ha, rodeadas de 54.250 Ha de Parque Natural en áreas adyacentes y 1500 de Preparque Marítimo en el litoral del Golfo de Cádiz. El PND se ha ampliado en 3.531 Ha en su margen nororiental según Resolución de 6 de febrero de 2004, de ampliación del PND (BOE núm. 47, de 24 de febrero de 2004).

Climatología. El clima de la zona se puede encuadrar dentro del tipo Mediterráneo subhúmedo con influencia atlántica, caracterizado por un invierno suave y lluvioso seguido de un periodo estival seco y por un régimen de temperaturas suaves a lo largo del año.

Las temperaturas medias mensuales se sitúan entre los 9 º C de enero y los 25-27 º C de julio. Es decir, la oscilación no suele superar los 15 º como media anual y los 25 º durante el día. Los valores extremos por debajo de 0 º son raros.

La zona se sitúa entre las isoyetas de los 500-600 mm anuales, con un promedio en la zona de la Reserva Biológica de 537 mm calculados en una secuencia de 23 años desde 1978 (www.rbd_csic.es). El máximo de precipitaciones se registra generalmente en invierno, seguido de las estaciones intermedias (otoño principalmente), mientras que en verano son bastante reducidas. La irregularidad interanual de las lluvias es otra de las características de la región. Frente a años de precipitación abundante se suceden períodos de sequía, suponiendo éstos últimos graves riesgos para el equilibrio ecológico del parque (Herrera, 1985).

Geomorfología y paisaje. Con un origen en el Cuaternario, Doñana es consecuencia de fenómenos de sedimentación y erosión provocados por el avance y posterior retroceso del mar sobre la costa, coincidiendo con los periodos de cambios climáticos, y que han conformado el característico paisaje dunar de Doñana junto con un relieve general suave, prácticamente plano en una primera aproximación. Sólo encontramos una topografía más abrupta en la zona costera donde las dunas se destacan sobre el resto del paisaje, alcanzando una altura alrededor de los 47 m (Loma del Chocolate).

Aguas freáticas. La estructura de la vegetación y su dinámica está íntimamente ligada al sistema freático existente en la zona (Muñoz-Reinoso, 2001).

Las aguas freáticas de Doñana pertenecen al sistema acuífero Almonte-Marismas, acuífero 27. Tiene una extensión de unos 3.400 km^2 según la FAO (1972) o de 2.400 km^2 en el estudio de IGME (1984). Geológicamente este acuífero, de edad Pliocuaternaria, se enmarca en el arco sedimentario Guadiamar-Guadalquivir. Limita al Este por los ríos Guadiamar y Guadalquivir, al Oeste por el Tinto, al Norte con

unos afloramientos de margas azules impermeables del Tortoniense y el Océano Atlántico al Sur y Suroeste (Custodio and Palancar, 1995).

En el PND la recarga del acuífero tiene su origen en la infiltración directa del agua de lluvia. Coincide en líneas generales con la topografía de la superficie del terreno. La principal modificación de la superficie freática es debida a las extracciones en los pozos de las zonas agrícolas y de abastecimiento.

La descarga del acuífero está dirigida por la existencia de un domo piezométrico al Oeste, en la zona del Abalario. El mar, el arroyo de la Rocina y los afloramientos del ecotono de la Vera son, junto con la evapotranspiración de la vegetación en las áreas donde el nivel freático es poco profundo y el flujo lateral lento a través del acuitardo, bajo la marisma, las principales direcciones de descarga. En las zonas de dunas la descarga se produce en dos frentes, hacia los corrales donde forma lagunas y charcas temporales, y hacia los cotos y marisma, conformando la hilera de lagunas permanentes o semipermanentes paralelas al frente dunas (Custodio and Palancar, 1995).Los cotos presentan zonas donde el elevado nivel freático intercepta o queda cercano a la superficie, dando lugar a zonas encharcadas, frente a zonas más altas que, debido a la alta permeabilidad de las arenas, son áridas. Este especial sistema se traduce en cambios de tipos de vegetación (Muñoz-Reinoso 2001).

Vegetación. Cada ecosistema en Doñana se va a caracterizar por una vegetación propia, originada por la combinación de diversos factores ambientales como son la salinidad, la topografía, el sustrato arenoso y el sustrato arcilloso. El gradiente salino y el gradiente de humedad (aridez/encharcamiento) junto con el grado de movilidad y asentamiento de las arenas modelan la vegetación (Zunzunegui et al. 1998; Muñoz-Reinoso, 2001). Entre las unidades ecológicas que se pueden describir destacan por el interés en este trabajo las dunas y cotos integrantes de las arenas.

Hacia el interior, la morfología actual es suavemente ondulada, encontrándose las mayores alturas al oeste del Parque (Naves), que van disminuyendo hacia la marisma. Ahora es la disponibilidad de agua el determinante de la vegetación de las arenas estabilizadas a tres escalas diferentes: a pequeña escala, distinguiendo las crestas dunares (monte blanco xerofítico) frente a los valles dunares (monte negro); a escala intermedia, distinguiendo las Naves (sabinar-monte blanco de *Rosmarinus officinalis*) frente al Manto arrasado (alcornocal-monte blanco de *Halimium halimifolium*); y a escala regional, distinguiendo la Vera frente al resto (Muñoz-Reinoso, 2001). La profundidad del nivel freático es, por tanto, uno de los factores determinantes de la distribución de especies.

Conviene destacar por su importancia en el presente trabajo las dos formaciones más características de este sistema de dunas estabilizadas. Por un lado, el **monte negro** que está dominado por los brezos *Erica scoparia*, *E. umbellata* y *Calluna vulgaris*. Se desarrolla bien sobre arenales profundos estabilizados que tienen la capa freática en invierno a menos de 40 cm. de la superficie del suelo. Estos brezales

representan una etapa de degradación avanzada del alcornocal con acebuches, en la que la materia orgánica se encuentra en estado muy ácido e inerte. Por el contrario, se conoce como **monte blanco** la formación vegetal con predominio de jaguarzo blanco o *Halimium halimifolium* que aparece acompañado de *Juniperus phoenicea*, *Rosmarinus officinalis, H.conmutatum, Cistus libanotis* en función del grado de humedad del suelo. Se desarrolla en suelos arenosos estabilizados con capa freática a mas de 40 cm. (Zunzunegui et al. 1998).

En la zona norte del PND la vegetación en las arenas estabilizadas fue además arrasada en la década de los 60 y sustituida por plantaciones de eucaliptales para obtención de celulosa (Granados, 1987). La política de conservación del PND ha llevado a cabo la eliminación completa de eucaliptos, dejando sólo algunos ejemplares aislados imprescindibles para la nidificación de rapaces.

LA PROTAGONISTA: PLEBEJUS ARGUS

1. Biología de la especie. *Plebejus argus* (Linnaeus, 1758) es un ropalócero de pequeño tamaño (24-28 mm. de envergadura) perteneciente a la familia Lycaenidae que tiene un marcado dimorfismo sexual. El macho es de color azul brillante, con una banda oscura terminal en las alas y un reverso alar blanco azulado claro y con una serie de puntos posdiscales negros, rodeados de blanco y puntos subterminales oscuros envueltos por una línea vestigial. El ala posterior se diferencia por la mancha interior azul metalizada en los puntos subterminales (Fernández Rubio, 1991). La hembra es menos llamativa, de color pardo y con el mismo patrón alar de puntos.

a) b) c)

Foto 1 a) Cópula de *P. argus* sobre gramínea. La coloración del macho, más azulada, contrasta claramente con la de la hembra de tonalidades marrones. b) larva de *P. argus* con las típicas bandas negras longitudinales. c) Pupa de *P. argus*.

Se trata de una mariposa univoltina, aunque en algunos lugares de Europa central se han descrito dos generaciones al año (Higgins and Riley 1973). El periodo de vuelo se extiende desde abril a julio variando según las latitudes (Brooks and Knight 1982), siendo más temprano en las zonas más meridionales, como Doñana, donde vuela entre finales de mayo y principios de julio. Las diferencias fenológicas de la especie en Doñana con respecto a otras zonas pueden deberse tanto a temperaturas inferiores en las zonas más septentrionales, como a las mayores altitudes a que en otros casos se hace referencia (Rodríguez et al. 1991 a).

Los machos aparecen algunos días antes que las hembras. La esperanza de vida media es de 3 o 4 días, aunque algunos individuos pueden llegar a tres semanas. Los adultos forman colonias a veces de muy alta densidad. Dependiendo de la latitud la puesta se produce en junio y principios de julio (Rodríguez et al. 1991) o más adelante en regiones más septentrionales. La presencia de hormigas es utilizada como indicadoras de buenos lugares de ovoposición (Pierce and Elgar 1985; Rodríguez et al. 1991a).

Los huevos son puestos muy cercanos al suelo desnudo, buscando un microclima más cálido durante la eclosión de las larvas en abril y mayo, favoreciendo así su desarrollo. Aparecen dispuestos individualmente sobre restos vegetales (hojas, ramitas...) de la planta nutricia (Jordano and Thomas,

1992), siempre lo más cerca posible de la base de la planta. En Doñana, Rodríguez et al. (1991a), señalan que, en su mayor parte, los huevos están en un radio de no más de 30 cm. de la base.

La larva pasa el invierno completamente desarrollada en el interior del huevo. Al eclosionar se mueve activamente en busca de la planta nutricia, y puede sobrevivir hasta 6 días sin comer (Emmet and Heath 1990). El desarrollo larvario dura alrededor de tres meses, durante los cuales se alimentan de hojas (flores en algunos casos) durante el día y la noche. Las carcasas no son comidas tras las sucesivas mudas (Brooks and Knight, 1982). Hay 4 estadios larvarios al final de los cuales alcanza alrededor de 13 mm de longitud (Emmet and Heath, 1990), aunque otros autores señalan hasta 5 estadíos (Rodríguez et al. 1991a).

Las crisálidas son alargadas y redondeadas, no angulosas. Miden de 8-9 mm de longitud. Tienen una depresión dorsal acusada entre el tórax y el abdomen y una estructura alar visible. De coloración ocre al principio, adquieren rápidamente una tonalidad verde.

La pupa se forma en la base de la planta nutricia, y permanece sin sujeción sobre una plataforma de seda (Brooks and Knight, 1982). En Doñana la pupa aparece dentro de los hormigueros de *L. niger* formados entre las raíces del *H. halimifolium*, sujeta por hilos de seda a las paredes del mismo (Rodríguez et al. 1991a). En algunos estudios se ha visto que las hormigas se sienten atraídas por las pupas y las atienden en el interior del hormiguero (Emmet and Heath 1990).

El adulto emerge tras un período de dos semanas y media (Brooks and Knight 1982) a tres semanas (Emmet and Heath 1990). Los adultos recientes parecen ser reconocidos por las hormigas y no son atacados mientras despliegan sus alas.

2. Relación mutualista. *Plebejus* mantiene una estrecha relación mutualista con hormigas del género *Lasius*: *L. alienus* (Emmet and Heath 1990) y *L. niger* (Rodríguez et al. 1991a). En una serie de estudios que se realizaron durante los años 80 y 90, se observó que la asociación entre *Lasius* spp. y *P. argus* era consistente a varias escalas espaciales en diversas partes de Europa (Thomas 1985; Ravenscroft 1990; Rodríguez et al. 1991a; Jordano et al. 1992; Jordano and Thomas, 1992). En el PND, se ha observado que los estadios tempranos (huevo, oruga y pupa) de *P. argus* están asociados de forma muy significativa con aquellas plantas de *H. halimifolium* que tienen un hormiguero de *L. niger* en la base (Rodríguez et al. 1991a). Así mismo, en áreas tan diversas ecológicamente como el norte de Gales, el sur de Inglaterra y Doñana, se ha observado que la densidad de adultos de *P. argus* está correlacionada positivamente con la densidad de nidos de *L. niger* y no con la de sus plantas huéspedes (Jordano et al. 1992; Jordano and Thomas, 1992). Todos estos resultados apuntan a que la relación mutualista de *P. argus* con *Lasius* spp debe tener una importancia crucial a la hora de explicar la calidad de hábitat de esta mariposa.

No obstante, la especificidad de *P. argus* es mucho mayor si se considera a una escala regional, no geográfica. Por ejemplo, en el PND, las orugas están asociadas preferentemente con una sola planta huésped, el jaguarzo *H. halimifolium*, y con una única especie de hormiga, *L. niger* (Rodríguez et al. 1991a), aunque aparece también de forma marginal sobre otras especies del matorral de Doñana como *Armeria velutina*, *Rosmarinus officinalis* (Rodríguez, 1991) siempre en relación con hormigueros de *L.niger*.

Larva y hormiga parecen responder en sincronía ante factores externos, posiblemente temperatura, a lo largo del día (obs.pers). A partir del ocaso las larvas suben por los tallos desde el hormiguero en la base hasta los brotes más tiernos en el extremo de la planta, donde permanecen rodeadas de hormigas alimentándose durante toda la noche. Por la mañana conforme aumentan las temperaturas las larvas descienden rápidamente hacia la base disminuyendo al mismo tiempo la actividad del hormiguero. Algunas larvas, de los primeros estadíos, permanecen en el exterior protegidas por la sombra de las hojas más jóvenes. Las larvas, además, han desarrollado un mecanismo de defensa consistente en dejarse caer al suelo ante cualquier movimiento brusco de la rama. Una vez en el suelo caminan hacia el hormiguero hasta encontrar hormigas que la llevan al interior. Este mecanismo podría haberse desarrollado como respuesta a la "predación inconsciente" por parte de herbívoros ungulados. Jordano y Thomas (1992) observaron además como las hormigas recogen las larvas de *P.argus* y las llevan al interior del nido. Las larvas en el interior del hormiguero también pueden recibir protección contra el fuego y temperaturas extremas (Emmet and Heath 1990).

	Enero	Febrero	Marzo	Abril	Mayo	Junio	Julio	Agosto	Septiembre	Octubre	Noviembre	Diciembre
Huevo												
Larva												
Crisalida												
Adulto												
Huevo												

Tabla 1 Fases del ciclo de vida de *P.argus* en Doñana según Rodríguez, 1991.

3. Plantas nutricias. En general, la polifagia es un carácter que suele estar asociado al mutualismo con hormigas dentro de la familia Lycenidae (New 1993). *P. argus* no es una excepción y se trata de una especie polífaga, que se alimenta de una gran variedad de plantas nutricias de la familia *Leguminosae*,

Ericaceae y *Cistaceae* (Thomas, 1985a; Rodríguez et al. 1991a). Aunque polífaga es localmente monófaga, adaptada a especies particulares por su estrecha relación con las hormigas. Diferentes estudios muestran que las puestas de *P. argus* de diferentes poblaciones están asociadas de forma significativa con distintas especies de plantas o combinaciones de planta y microclima (Rodríguez et al. 1991a; Thomas and Lewington 1991).

En realidad hay casi tantas asociaciones con plantas como poblaciones estudiadas. Tal es el caso de *Ulex europaeus y Lotus corniculatus (*Brooks and Knight 1982), *Trifolium spp., Astragalus spp., Colutea spp., Genista spp. (*Fernández Rubio 1991), *Calluna vulgaris y Helianthemum spp* (Emmet and Heath 1990) y por último *H. halimifolium* (Rodríguez, et al. 1991)

La planta nutricia fundamental de *P. argus* en Doñana (Rodríguez et al. 1991a) es *H. halimifolium* (L.)Wilk. Se trata de un arbusto muy ramoso, erecto, de hasta 2 m de altura, perteneciente a la familia de las cistáceas, típica de suelos arenosos, tanto litorales como del interior. Su distribución europea se centra en la cuenca mediterránea occidental, desde Italia hasta Portugal, estando presente también en el norte de África. En Andalucía occidental aparece en las Sierras de Aracena y norte de Sevilla y por toda la costa, desde la desembocadura del Guadiana hasta el estrecho de Gibraltar.

Es uno de los principales componentes de la vegetación de las arenas estabilizadas aunque aparece en todas las formaciones ecológicas, desde las depresiones inundables hasta las partes altas de las dunas fijas, siendo más abundante en las zonas intermedias donde domina en la composición del matorral (Zunzunegui et al. 1998).

LA HORMIGA: *LASIUS NIGER*

El género *Lasius* es uno de los más comunes en la fauna neártica. *Lasius niger* (Linnaeus, 1758) anida en suelos expuestos o bajo piedras y raíces. En Europa, ésta es la especie del género *Lasius* que se encuentra comúnmente en el entorno urbano (Seifert, 1992). Tiene una amplia distribución en la península ibérica, ligado a ambientes relativamente húmedos y cercanos a poblaciones humanas (Luque García et al. 2002).

L. niger se considera especie característica de etapas pioneras de la sucesión. Numerosos autores han seleccionado a hormigas de este género para el estudio de la distribución espacial de invertebrados terrestres en ambientes de este tipo (Boomsma and De Vries, 1980; Boomsma and Van Loon, 1982; Holec et al. 2006).

Foto 2 Reina de *L. niger* cuidando de la puesta.

El desarrollo larvario de las hormigas depende en gran medida de la temperatura. Es por tanto lógico esperar una asociación entre distribución de hormigueros y gradiente de temperatura, correlacionándose la densidad de poblaciones de *L. niger* con la temperatura de la cubierta de hojas, temperatura del suelo, humedad y contenido en agua del suelo (Galle, 1975).

En Doñana, caracterizada por los ciclos anuales de inundación/desecación y donde se alcanzan temperaturas extremas en el suelo en verano, la humedad puede ser un factor tan determinante como en los estudios de Boomsma et al. (1982).

El soporte físico de la estructura del hormiguero es de vital importancia en el caso de especies que vivan en ambientes extremadamente secos y adversos como es el caso que nos ocupa. La naturaleza arenosa del suelo de la zona se convierte en el factor limitante en la construcción de hormigueros y convierte las partes subterráneas de las plantas en soporte para la construcción de los hormigueros. La planta sirve pues de estructura física además de proporcionar un microclima más benigno en humedad y temperatura. El sombreado de la planta amortigua los valores extremos de temperatura y humedad. Además, a escasa profundidad por debajo de la superficie las oscilaciones diarias y anuales de estos factores tienden a amortiguarse todavía más.

L. niger no sólo responde a los cambios abióticos de forma espacial sino que parece existir una variable comportamental de la especie a las variaciones de temperatura. Las hormigas pueden presentar a lo largo del día ciclos periódicos de actividad que les permiten forrajear distintas partes de su hábitat a distintas horas del día o de la noche (Ballard and Pruess, 1980). *L. niger*, al igual que otras especies del mismo género como *L. neoniger*, es en época estival un forrajeador nocturno, cuando las temperaturas que se pueden alcanzar en el área de estudio a nivel del suelo pueden rondar los 60 º C. Otras especies, como es el caso de *Cataglyphis* sp. prefieren, por el contrario, forrajear en las horas del día de mayor temperatura (Cerdá and Retana, 2000) o modifican su comportamiento en función de la temperatura en la que forrajean(Doblas-Miranda and Reyes López, 2008). En Doñana, *L. niger* presenta picos de actividad en las horas en las que la temperatura es más benigna: crepúsculo y nocturnas en verano, mediodía en época invernal (obsv. pers.).

El género *Lasius* se relaciona con hasta 46 especies de licénidos en todo el mundo, dos de ellas de forma obligada (Fiedler, 1991). En Doñana, *P.argus* está íntimamente ligada a la presencia de hormigueros de *L. niger* y, por tanto, cualquier factor que afecte a la distribución de la hormiga va a afectar indirectamente a la distribución de la mariposa asociada. En las relaciones mutualistas obligadas, la asociación tiene lugar casi siempre con la misma especie de hormiga o al menos con el mismo género (Eastwood and Frase, 1999). En tales relaciones especializadas la presencia de la hormiga regula la distribución espacial y temporal de la mariposa asociada (Jordano et al. 1992; Seymour et al. 2003). Más aún, estas interacciones obligadas tienen lugar preferentemente entre especies de hormigas ecológicamente dominantes que forman colonias grandes y duraderas y que exhiben comportamientos territoriales. En este sentido, las hormigas del género *Lasius* se clasifican como dominantes de segundo orden según la clasificación de Fiedler (1991). Es decir, especies subordinadas a las especies dominantes en el hábitat, pero que pueden llegar a ser dominantes o abundantes en ausencia de éstas y van a monopolizar recursos del hábitat. Dentro de este grupo se incluyen numerosas especies dominantes en hábitat alterado como podría ser el caso de *Lasius*.

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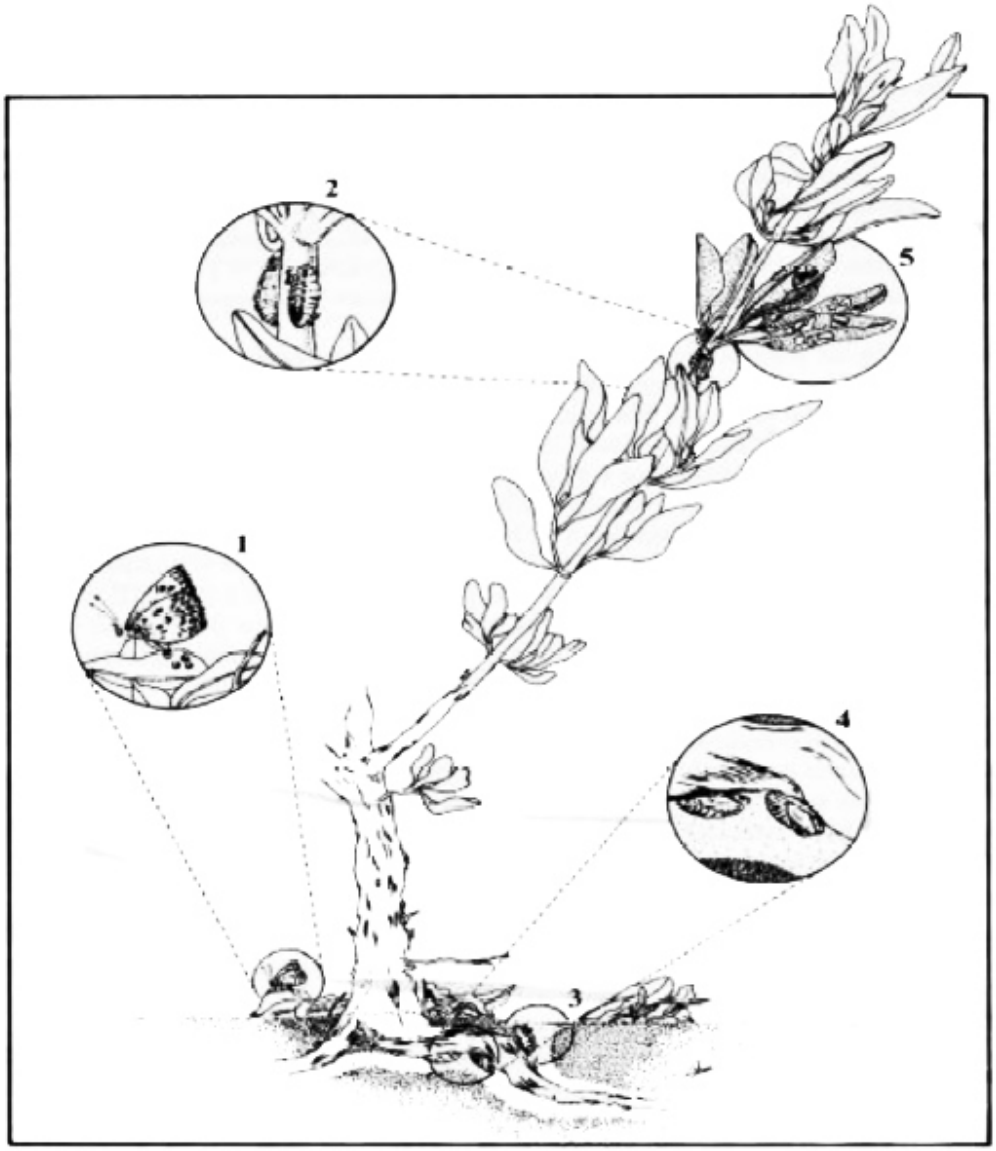

BLOQUE I.-*LA DISTRIBUCIÓN ESPACIAL DE P. ARGUS: DISTINTAS ESCALAS DE ANALISIS*

[CAPÍTULO 2: ESCALA NACIONAL]

"LIVING ON THE EDGE IN SPECIES DISTRIBUTION MODELS: THE UNEXPECTEDPRESENCE OF THREE SPECIES OF BUTTERFLIES IN A PROTECTED AREA IN SOUTHERN SPAIN"

"LIVING ON THE EDGE IN SPECIES DISTRIBUTION MODELS: THE UNEXPECTED PRESENCE OF THREE SPECIES OF BUTTERFLIES IN A PROTECTED AREA IN SOUTHERN EUROPE"

Resumen

El método de modelado MaxEnt (Máxima entropía) es probablemente la técnica más popular para modelar distribuciones de especies basadas en registros de presencia a lo largo de escalas espaciales amplias. Aunque es ampliamente utilizado existe mucha controversia acerca de la transferencia de los modelos entre diferentes áreas geográficas. La trasferencia podría ser más cuestionable aun cuando se intenta predecir la distribución de poblaciones periféricas en los márgenes del rango geográfico de las especies donde pueden verse afectadas y estar adaptadas a condiciones ambientales diferentes de las poblaciones centrales. Para explorar la transferencia de modelos de MaxEnt entre sectores del rango geográfico, seleccionamos tres especies de mariposas con una amplia distribución y con poblaciones periféricas en el margen más al sur de la península ibérica, *Plebejus argus, Cyaniris semiargus y Pironia tithonus.*

Mediante el uso de datos del Atlas de las mariposas de la Península ibérica e Islas Baleares y datos de clima y usos del suelo, modelamos sus distribuciones potenciales en España. Además también investigamos la transferencia de los modelos entre sectores y el efecto de variar el parámetro de regularización. Nuestros resultados muestran que cuando se desarrollan modelos de distribución la calidad de los datos de presencia debe ser cuidadosamente testado, prestando especial atención tanto al número como a la distribución espacial y evitando posibles sesgos significativos.

La transferencia de los modelos tiende a decrecer cuando se usan datos de sectores cada vez más distantes como datos de test. Más aun, e independientemente del parámetro regularizador, los modelos construidos con datos de presencia del sector central o intermedio fueron incapaces de predecir adecuadamente la distribución de las tres especies de mariposas en el sector periférico, especialmente en el Parque Nacional de Doñana.

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Living on the edge in species distribution models: The unexpected presence of three species of butterflies in a protected area in southern Spain

Pilar Fernández*, Diego Jordano, Juan Fernández Haeger

Department of Botany, Ecology and Plant Physiology, Universidad de Córdoba, Campus de Rabanales, Edificio C-4, 14071 Córdoba, Spain

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ABSTRACT

MaxEnt (Maximum Entropy) modelling method is probably the most popular technique to model species distributions based only on the presence records across broad spatial scales. Although it is widely used, there is much controversy about the transferability of models between different geographical areas. Transferability might be more questionable when it comes to predict the distribution of peripheral populations at the margin of the species geographical range, where they may be affected by and adapted to environmental conditions different from those of core populations. To explore transferability of MaxEnt models among sectors of the geographic range, we selected three butterfly species with wide distributions and peripheral populations at their southernmost margin in the Iberian Peninsula, namely *Plebejus argus*, *Cyaniris semiargus* and *Pyronia tithonus*.

Using data from the Atlas of the butterflies of the Iberian Peninsula and Balearic Islands as well as both climate and land use data, we modelled their potential distribution ranges in Spain. In addition, we also independently modelled their distributions separately in three concentric sectors of their range. We then investigated the transferability of the models between sectors and the effect of varying the regularization parameter.

Our results show that when developing species distribution models the quality of occurrence data should be carefully checked, paying special attention to both their number and spatial distribution and avoiding possible significant biases.

The transferability of the models tends to decrease when data from increasingly distant sectors are used as test data. More precisely, and independently of the regularization parameter value, models built using occurrence data either from the core or the intermediate sectors failed to adequately predict the distribution of the three butterfly species in the peripheral sector, especially in Doñana National Park.

1. Introduction

Species distribution models (SDMs) are specific tools developed for predicting the habitat and potential distribution of plant and animal species given a set of occurrence data, albeit incomplete, and environmental data. SDMs have acquired increasing importance in biodiversity conservation (Fielding and Bell, 1997; Araújo and Luoto, 2007; Mateo et al., 2011), and to exploring the potential effects of global climate change on biodiversity loss and on shifts in species distributions (Pearson and Dawson, 2003; Elith et al., 2010; Romo et al., 2013.)

A variety of modelling techniques ranging from classic logistic regression models coupled with GIS, generalized additive models (GAM), GARP (a genetic algorithm approach) and others, are available (Virkkala et al., 2005; Wisz et al., 2008; Titeux et al., 2009). Some authors focus on modelling the environmental conditions that meet a species' ecological requirements and predict the relative suitability of habitat, aiming to produce the so called environmental niche models (ENMs) (Warren and Seifert, 2011). In practice, when modelling across large geographic areas there is usually a lack of data concerning important niche dimensions linked to biotic factors, while detailed data of climatic variables, altitude, slope, or aspect are more easily available.

In any case, the model results are often flawed by problems like small sample sizes, biased data or unrepresentative samples (Dennis and Thomas, 2000; Stockwell and Peterson, 2002; Romo et al., 2006; Pearson et al., 2007; Papeş and Gaubert, 2007).

* Corresponding author. Tel.: +34 957218596.
E-mail address: bv2ferop@uco.es (P. Fernández).

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Additional problems are lack of real absences, or the choice of variables to be included (Araújo and Luoto, 2007; Titeux et al., 2009). Moreover, the ecological characteristics of the target species may have potential effects on the model output (Osborne and Suárez-Seoane, 2002; Mcpherson et al., 2004).

MaxEnt is a maximum entropy modelling method that performs extremely well in predicting occurrences, especially when sample size is small (Phillips et al., 2006; Pearson et al., 2007; Wisz et al., 2008).

On the other hand, transferability or the ability of a model calibrated in one context to make useful predictions in a different context, has also been the centre of several papers and the focus of discussion (Lobo et al., 2007; Peterson et al., 2007; Phillips, 2008). However, the environmental envelope of a given species may drastically change across its geographic range from the core area to the edges due to spatial heterogeneity. Under these circumstances, a model cannot always include the full spectrum of conditions of the target species if its range is not well sampled. In cases where the sampling distribution is expected to be strongly biased, its negative effect on the model's accuracy can be minimized in MaxEnt by targeting background locations from heavily sampled areas to provide unbiased results (Phillips et al., 2009).

The spatial patterns shown by many species across their ranges can also compromise the predictive ability of MaxEnt models. Near the core of the distribution area local populations tend to be bigger in size, more abundant and experience a more suitable environment than the more fragmented, peripheral populations occupying less favourable habitats near the edge of their geographic distribution, (Brown, 1984; Lawton, 1993) (but see Sagarin et al., 2006; Munwes et al., 2010). Moreover, contrary to initial expectations peripheral populations have been shown to be adapted to the edge of their range (Zakharov and Hellmann, 2008), can persist as well as core populations (Channell and Lomolino, 2000), and may have different genetic traits and more genetic diversity than core populations (Munwes et al., 2010). For these reasons, differences in population numbers, extent and size from core to peripheral areas can lead to misinterpretation of the predicted habitat through SDMs. However, this problem has received little attention.

This could be the case of *Plebejus argus*, *Cyaniris semiargus* (Lycaenidae) and *Pyronia tithonus* (Satyridae), three butterfly species widely distributed across the European continent. Within the Iberian Peninsula they have their core areas in northern Spain, where they occur in almost every 10×10 km UTM square (Universal Transverse Mercator conformal projection), showing a rather continuous distribution. However, as we move to the drier and warmer southern margin of their range, their distributions gradually become more fragmented with increasingly isolated local populations.

Unexpectedly, these three species occur in the Doñana national park (southern Spain) almost at sea level at the southern margin of their continental range, despite the high values of solar irradiance and temperatures and the scarce annual rainfall (Fernandez Haeger et al., 1976). The nearest populations at similar latitude occur in the mountains at much higher elevations.

The maximum predictive accuracy of models may not be independent of range size (Stockwell and Peterson, 2002), and therefore widespread species like these could be modelled less accurately. In these cases, it has been suggested that model accuracy can be improved by splitting species distributions into sub-units or sectors that may have different ecological characteristics (Osborne and Suárez-Seoane, 2002; Thuiller et al., 2004).

The main objective of this work is to model the current potential distribution of the butterflies *P. argus*, *C. semiargus* (Lycaenidae) and *P. tithonus* (Satyridae) in Spain. More specifically we divided the range of each species into three different concentric sectors namely core, intermediate and peripheral, starting at the core area for each species in Spain. We then produced a set of models using occurrence data from the whole extent of peninsular Spain (global model at national scale) and a separate model for each sector independently, and tested the transferability of the global model to each sector and vice versa. This approach is aimed to test the performance of modelling on core versus marginal populations. We hypothesize that model transferability among sectors will be low, decreasing as the distance between sectors increases. Secondarily, we compare alternative models to test the effects of controlling bias and overfitting and evaluate variable contribution.

2. Methods

2.1. Data extraction and sectorization

Presence data of the three species were obtained from the *Atlas of the butterflies of the Iberian Peninsula and Balearic Islands* (García-Barros et al., 2004). This is the most comprehensive review up to date of butterfly distributions in Spain. Although it compiles a large dataset from museums, collections and published records spanning from the 19th century to the present, most of the data belong to the period 1975 onwards. The spatial reference is the 10×10 km grid of the Universal Transverse Mercator conformal projection (UTM). The available occurrence data for the three butterfly species totals 1080 UTM squares for *P. tithonus*, 670 for *P. argus* and 399 for *C. semiargus*.

Using ArcGis 10.1 (ESRI, 1998) we mapped the occurrence records for each species in UTM grid (Fig. 1). ArcGis tools allowed us to find for each species the centroid or geometric centre of the occupied squares, as well as to compute the distances from these squares to the centroid and the standard deviation. For each species we divided the geographical extent into a core, a medium and a marginal or peripheral sector (Fig. 1). These three sectors are concentric relative to the centroid of the distribution, with radiuses equal, respectively, to one, two and three times the standard deviation of the mean distance from the centroid to the occupied UTM grid squares (Osborne and Suárez-Seoane, 2002). The underlying assumption for this decision is that environmental conditions change with the distance from the distribution core. The frequency of occurrence data by species and sector (core, intermediate and marginal) were as follows: *P. tithonus* 678, 375 and 27 UTM squares, respectively; *P. argus* 447, 202 and 21 UTM squares, respectively, and *C. semiargus* 285, 99 and 15 UTM squares, respectively.

2.2. Assessing bias

The available occurrence data (museum collections, atlases, etc.) often exhibit strong spatial bias in survey effort (Dennis and Thomas, 2000; Romo et al., 2006; Phillips et al., 2009; Lehikoinen, 2013). This bias can severely impact model quality and results (Elith et al., 2010; Kramer-Schadt et al., 2013). To control for sampling bias effects in our models we built ordinary MaxEnt models with default settings (MaxEnt 3.3.3.k version) as controls and also modified models including two types of bias files, each of which constitutes a background sample with the same biased error as the occurrence records (Phillips et al., 2009).

The first bias file (hereafter the target group bias, Fig. 2) was generated by pooling the occurrence data of the three butterfly species and assigning a value of 1 to every UTM square with at least a positive record of any of the three butterfly species, and −999 to squares without any single occurrence record. This approach is based on the idea that a specialist visiting a given locality or UTM square would register all the butterfly species he/she might encounter, so the absence of one species where others were present could be

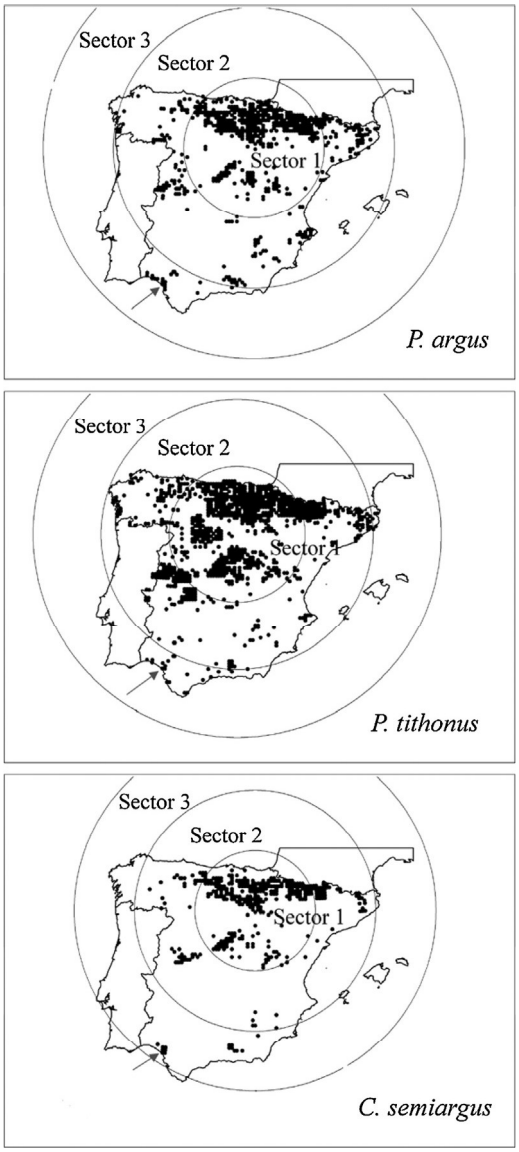

Fig. 1. UTM 10 × 10 km squares with recorded presence of the different species and the concentric sectors distinguished. The arrow shows the location of Doñana national park.

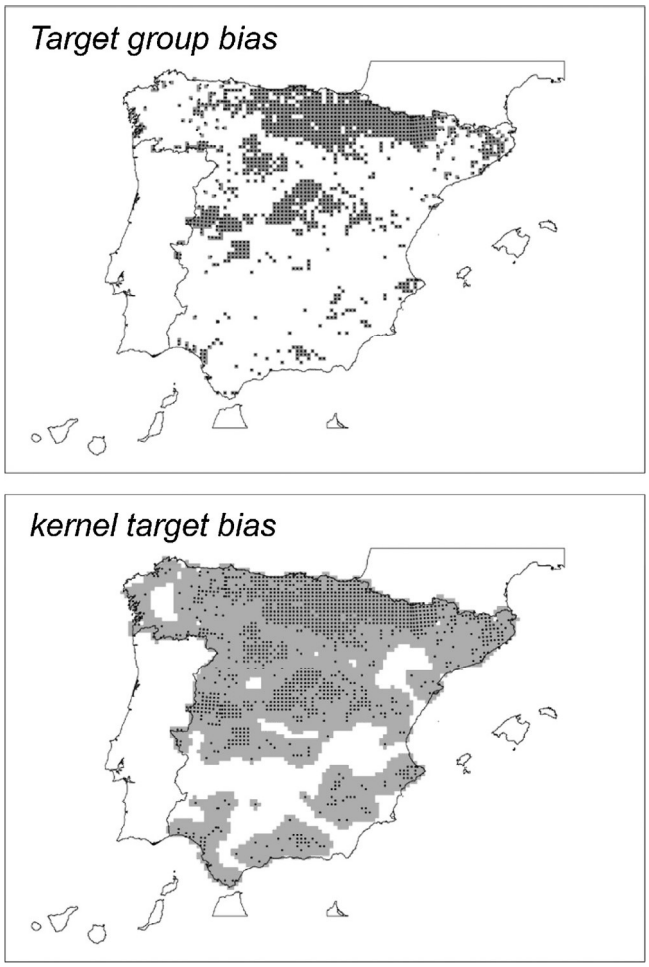

Fig. 2. Bias used in models. Points represents presences of the three species pooled while grey area in kernel target bias is the complete area include during modelling.

considered as a real absence (Elith et al., 2010; Mateo et al., 2010). MaxEnt was then forced to select background points from the subset of UTM squares with a value of 1.

The second bias file (hereafter the kernel bias, Fig. 2) was elaborated using a kernel smoothing technique (Horne et al., 2007; Elith et al., 2010) with Arcgis to calculate the density of presences in a neighbourhood around the grid cells with occurrence data of any of the three species (ESRI, 1998). Subsequently we reclassified the grid cells assigning a value of 1 to any UTM square showing at least the same minimum kernel density as an "occupied" UTM square, and a value of −999 to the rest of squares.

2.3. Predictive variables used

MaxEnt works by finding the largest spread (maximum entropy) in a geographic dataset of species presences in relation to a set of predictive, 'background' environmental variables. Climate is considered the main cause of species distribution at a broad spatial scale (Parmesan, 1996; Guisan and Zimmermann, 2000; Pearson and Dawson, 2003; Thuiller et al., 2004; Rahbek et al., 2007; Romo et al., 2013). However, the suitability of the environmental conditions for a target species can also be related to a number of non-climatic factors (Pearson et al., 2004; Araújo and Luoto, 2007; Luoto et al., 2007; Gutiérrez et al., 2005; Heikkinen et al., 2007; Titeux et al., 2009).

The climatic and altitude values were obtained from Worldclim (Hijmans et al., 2005). WorldClim is a set of global climate layers generated by interpolation of data gathered by weather stations worldwide during the period 1950 to 2000, plus a Global Digital elevation model covering the whole Earth. The dataset includes 19 bioclimatic variables, of which we selected the following 14 variables according to their ecological significance and potential predictive power as shown in other studies: mean annual temperature (t_mean), maximum temperature of warmest month (t_max), minimum temperature of the coldest month (t_min), annual precipitation (p_mean) and precipitation of the driest month (p_driest), precipitation of the wettest month (p_wettest), precipitation of wettest, driest, warmest and coldest quarter and mean temperature of wettest, driest, warmest and coldest quarter. The 1 km^2 resolution layer of WORLDCLIM was re-scaled to the working resolution of 10 × 10 km, calculating the mean values of all variables for each UTM square.

Land use variables were obtained from the CORINE Land Cover 2006 (European Environment Agency, 2012). This layer classifies land use into a number of classes that for the sake of simplicity we reduced to ten: inland water, inland wetland, "maritiwater", "maritiwetlands", arable land, heterogeneous

agricultural areas, permanent crops, pastures, artificial forests and shrub and/or herbaceous areas. The percentage of area occupied by each class of land use in the 10 km UTM squares was calculated using Arcmap 10.1. Additionally, the area of urban and protected areas was obtained from the Europarc network (http://www.redeuroparc.org).

2.4. Reducing multicollinearity

The independence of the predictor variables affects the stability of the model (Muñoz and Felicísimo, 2004; Merow et al., 2013). To check for multicollinearity we computed the Spearman's correlation index among predictive variables across the whole geographic extent covered in this study, eliminating correlated variables where Spearman's $r \geq 0.7$. As a result, we finally selected as non-climatic variables altitude, percentage of urban area, protected area, agricultural area, permanent crops, pastures, heterogeneous agricultural areas, shrub and/or herbaceous areas, wetlands and water units. Among the climatic variables we selected: mean annual temperature, maximum temperature of the warmest month, annual precipitation, precipitation of the driest month, minimum temperature of the coldest month, and precipitation of the wettest month.

2.5. Models tested in the same area: Evaluation of bias and contribution of variables

We developed three alternative approaches using different MaxEnt settings, the first was to build a simple model as control without using a bias file, the second was a model using the target group bias file, and a third model using the kernel bias file. The bias files have already been described under the subheading "assessing bias". These three alternative models were built for each species at four spatial scales: national (global) or the peninsular territory of Spain, the core area of distribution in Spain, the intermediate sector of the distribution area, and finally the marginal or peripheral sector. In each case the model was built using a mask to select occurrence and background data only from the area being modelled, and then projecting the model to the whole peninsula. The flow diagram in Fig. 3 tries to summarize the components and levels that were combined to built the models.

MaxEnt is prone to overfitting especially when presence data are scarce and too many environmental variables are included in the model. While some authors have shown that default settings perform as well as adjusted settings (Phillips, 2008), others have pointed that when transferability is a matter of concern it is recommended to evaluate models using different values of the regularization parameter beta (β). We tested different models using six different values of β: 0.1, 0.5, 1, 3, 5 and 9. In each case 15-fold cross validation were used per model.

The 15 replicates of each model were averaged to obtain a single predictive map. To measure the effectiveness of these predictive models, we used a subsample of occurrence and background data from each sector. Whenever possible, each subsample included a 25% of presence data selected at random, as well as 100 background data. For all the three species occurrence data in the peripheral sector were relatively scarce, for this reason the subsample dataset included a minimum of 6 to 10 presences and 100 background data randomly selected.

Models were evaluated using both threshold-independent and threshold-dependent metrics that assess various aspects of performance and overfitting. The threshold-independent metrics derive from the Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) plot. First, we selected the ROC created by plotting the sensitivity values against 1-specificity for all the available probability thresholds (Fielding and Bell, 1997; Manel et al., 2001). It can also be generated with presence and background absence data (Phillips et al., 2006) and the resulting AUC tests the ability of the algorithm to discriminate between a grid cell with suitable environmental conditions and a random grid cell (background) (Phillips et al., 2006). The ROC curves were plotted and the AUC value calculated in SPSS. Second, we used AUC difference (AUC_{diff}): $AUC_{calibration}$ minus $AUC_{evaluation}$. A smaller AUC_{diff} entails a lesser overfitting in the model (Warren and Seifert, 2011).

In addition, a variety of error measures can be calculated from the confusion matrix (Manel et al., 2001) that shows the results of actual occurrence data (positive or negative) in columns, and the predicted occurrences (positive or negative) in rows. The four resulting classes are: ++) TP or true positive; +−) FP or false positive; −+) FN or false negative and −−) TN or true negative. The classification results were used to compute 'sensitivity', the conditional probability that a positive case is correctly classified, and Cohen's Kappa index which takes into account the whole confusion matrix (see Fielding and Bell, 1997 for more details). These evaluation methods require a threshold to transform continuous model predictions to dichotomous classifications of presence/absence. For this purpose we selected equal training sensitivity and specificity. This value was determined for each model and averaged over the 15 replicate models to obtain the threshold for each species and model.

National and sectorial models of the three species were used to assess the effects of bias and the contributions of predictive variables. Paired comparisons between models with different bias were analyzed with the Friedman's ANOVA test.

A GLM factorial ANOVA approach was used to test for differences in the relative contribution of variables (climate versus land use variables) to the models, considering the percent of contribution of each predictor (log + 1 transformed) as dependent variable. Meanwhile, sector (national, core, intermediate and peripheral), type of variable (climate or land use), species and β were considered factors.

2.6. Models tested outside: Evaluation of transferability

In order to test the transferability of a model built for a given sector (core, intermediate or peripheral) to a different sector, the model was projected to the whole peninsula, and the predicted occurrences and absences in the target sector were compared with a random subsample of the available occurrence data (25%) and 100 background data for that target sector. This allowed us to generate a confusion matrix and calculate sensitivity, Kappa and AUC values. In addition, validation tests for the national models were run on presences of each single sector and vice versa.

Sensitivity, Kappa and AUC values were calculated considering the 16 possible combinations of sector (national, core, intermediate and peripheral) taken as training and as test sectors. For the sake of simplicity we have considered four discrete distance steps involved in each model transfer: step = 0, both training and test data belong to the same sector (no transfer); step = 1, training and test data belong to two adjacent sectors; step 2 = training and test data belong to two sectors separated by a third sector (i.e. core versus peripheral sector); step = 3, training and test data belong to two sectors that are separated from each other by two intermediate sectors (i.e. national versus peripheral sector) models.

Transferability and relative performance of sectorial models were evaluated with a GLM factorial ANOVA applied to the resulting AUC, sensitivity and Kappa values as dependent variables, and step and β as factors. AUC sensitivity and Kappa were arcsin transformed previous to the analysis.

Finally, we tested the ability of all models to predict the occurrence of the three butterfly species in the peripheral sector, at the southernmost edge of the species range in Europe.

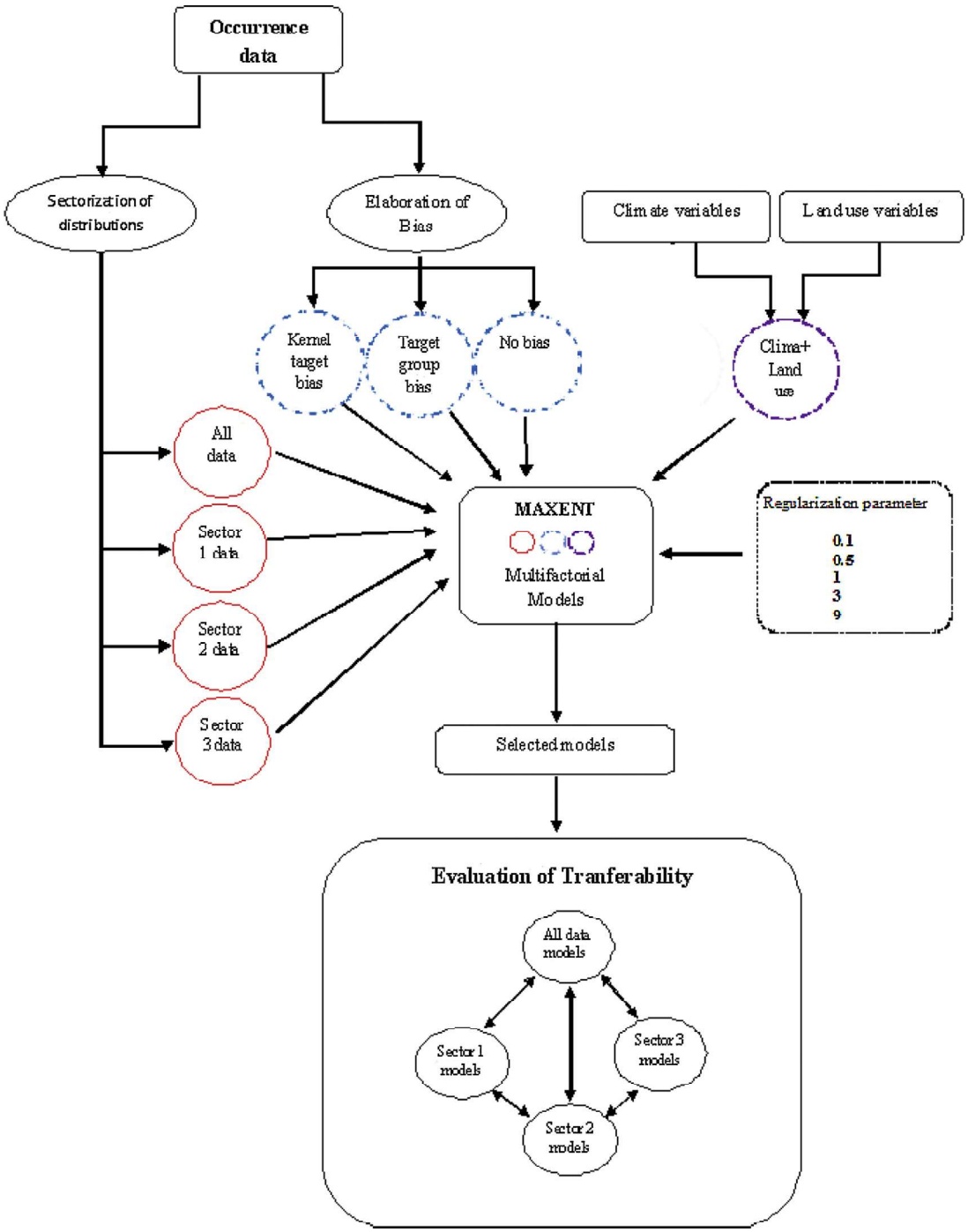

Fig. 3. Flow diagram showing the different factors involved in the modelling job.

3. Results

3.1. Models tested with the same data: Evaluation of bias and variable contribution

Results of AUC, Kappa and sensitivity values of models independently developed for each species at the national scale and for the core and the intermediate sectors, either using the kernel bias file or without any bias file (control models), gave acceptable values, higher than similar target-bias models (Table 1). However, measures of the three indexes in models for the peripheral sector were substantially lower. Besides, AUC, Kappa and sensitivity values from models using the target-bias approximation were lower than those obtained with kernel or control models without bias file, the two latter not being significantly different. Fig. 4 shows the behaviour of AUC_{diff} in the full spectrum of models. Overfitting in both kernel and control models was similar and increased with decreasing β values, as expected. Target-bias models yielded higher overfitting values regardless of the β value used.

Although control models performed as well as kernel bias models, we restricted our subsequent analysis to the kernel models.

The GLM type III factorial ANOVA showed that sector ($F_{3,\ 1105} = 8.33$ $p < 0.000018$), variable type – climatic or land use variables – ($F_{1,\ 1105} = 16.22$ $p < 0.00006$) and their interaction ($F_{3,\ 1105} = 21.45$ $p < 0.0000$) explained significantly the observed variance in variable contribution to the models. β and interactions

Table 1
Mean value and standard error of AUC, Kappa and sensibility in models generated with different bias files. Results of the Friedman's ANOVA by ranks (bias selection) are also shown.

		Bias type			
		Kernel	No bias	Target	ANOVA Chi Sqr. ($N = 18$. df = 2)
National	AUC	0.90 ± 0.008	0.91 ± 0.008	0.82 ± 0.02	29.6 $p = 0.00000^{***}$
	Kappa	0.64 ± 0.02	0.64 ± 0.02	0.48 ± 0.03	28.9 $p = 0.00000^{***}$
	Sensitivity	0.74 ± 0.01	0.76 ± 0.01	0.61 ± 0.02	34.7 $p = 0.00000^{***}$
Core	AUC	0.83 ± 0.013	0.84 ± 00.01	0.76 ± 0.04	12.19 $p = 0.002^{**}$
	Kappa	0.53 ± 0.02	0.55 ± 0.02	0.38 ± 0.05	25.44 $p = 0.00000^{***}$
	Sensitivity	0.64 ± 0.01	0.67 ± 0.007	0.51 ± 0.02	33.07 $p = 0.00000^{***}$
Intermediate	AUC	0.81 ± 0.01	0.82 ± 0.01	0.72 ± 0.02	31.01 $p = 0.00000^{***}$
	Kappa	0.43 ± 0.01	0.46 ± 0.01	0.35 ± 0.03	36.00 $p = 0.00000^{***}$
	Sensitivity	0.53 ± 0.02	0.58 ± 0.02	0.42 ± 0.02	36.00 $p = 0.00000^{***}$
Peripheral	AUC	0.52 ± 0.03	0.58 ± 17 0.04	0.39 ± 0.03	16.34 $p = 0.00028^{***}$
	Kappa	0.03 ± 0.02	0.08 ± 0.02	0.09 ± 0.03	16.00 $p = 0.00034^{***}$
	Sensitivity	0.18 + 0.03	0.2 + 0.046	0.22 + 0.03	72.950 $p = 0.00000^{***}$

$^{**}p < 0.01$, $^{***}p < 0.001$.

with β were not significant. In general, the contribution of climatic variables was higher than those of land use variables in all sectors excepting the peripheral sector.

In models trained at national scale, the relative contribution of climatic variables to the model (mean% ± SE: 12.33 ± 1.099) was higher than that of land use variables (2.60 ± 0.192.62), although the contribution of each individual variable varied among species (see Table 2). However, going from the national scale model down to core, the intermediate and the peripheral models, the weight of climatic variables tended to decrease, while the weight of the land use variables tended to increase.

In the core sector, climate variables contributed most to the models (mean ± SE: 12.37 ± 1.83), whereas the contribution of land use variables was lower (2.58 ± 0.22). In the intermediate sector, the contributions of climatic variables (mean ± SE, 6.52 ± 0.54) and land use variables (mean ± SE, 6.09 ± 0.58) were more balanced (see Table 2 for contribution of individual variables).

In the peripheral sector the mean contribution of climate variables was significantly lower (1.51 ± 0.25) than that of land use variables (9.1 ± 1.09), (see Table 2 for contribution of individual variables). Wetlands was the most important land use predictor, with contributions of 48.8% in the *P. argus* model, 19.7% in *C. semiargus* and 29.1% in *P. tithonus*. In general, the weight of non-climatic variables in models for the peripheral sector was greater than for models for the core and the intermediate sectors.

Fig. 5 shows the percentage contribution of all climatic variables versus land use variables for each species while Fig. 6 shows the potential distribution maps of the three butterfly species by sector, predicted by models using default settings and no bias file.

3.2. Models tested in each sector subsample: Evaluation of transferability

National scale models built using all the available presence data, performed reasonably well when they were transferred to the core, the intermediate or the peripheral sector (one, two or three steps Fig. 7). Increasing the value of β in the models reduced overfitting (Fig. 4), but yielded worse AUC, sensitivity and kappa results. In these models there was a significant effect of step in the observed values of AUC, sensitivity or kappa (GLM factorial Anova, factors arcsin transformed, Wilks lambda = 0.199 $F_{9,\ 114.54} = 11.93$ $p < 0.0001$), but neither betaβ nor their interaction had a significant effect β (Wilks lambda = 0.80 $F_{15,\ 130,15} = 0.71$ n.s). Applicability of the national model to any given sector of the distribution area decreased as distance from the centroid to the sector increased, but results differed slightly according to the β value used. National

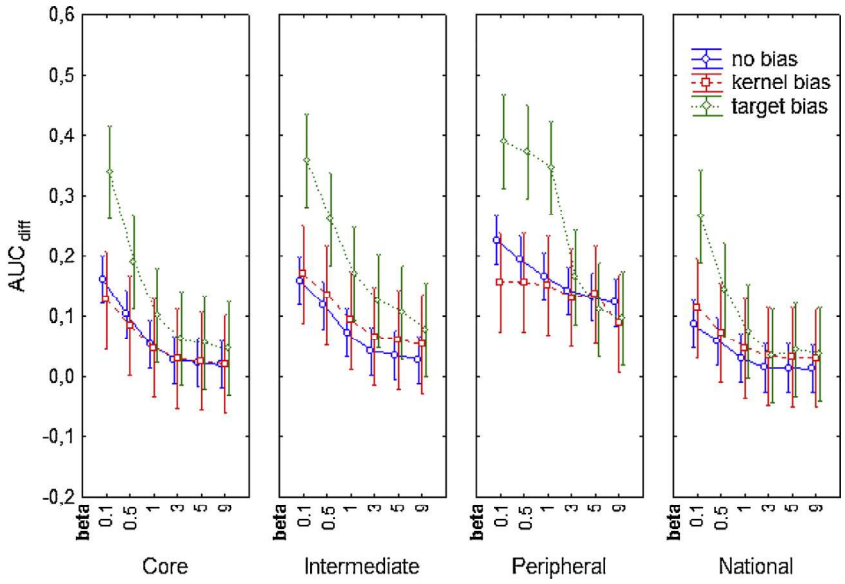

Fig. 4. Mean values and errors bars of AUC_{diff} according to model (core, intermediate, peripheral and national), type of bias file employed and β level.

Table 2
Contribution of climatic and non-climatic variables to the national and sectorial models. We show here only data from kernel models and default setting ($\beta = 1$). The contribution of each variable (%) is presented in brackets. Land use variables are in italics. Only contributions higher than 5% are shown.

	Kernel models $\beta = 1$		
	Plebejus argus	*Cyaniris semiargus*	*Pyronia tithonus*
National	Pdriest (33.6) Tmean (12.1) Pmean (13.7) Tmax (12.4) Pwettestmonth (7.3)	Tmean (45.7) Pdriest (18) Pmean (5.8) Tmax(6.2) Pwettestmonth (5.6)	Pdriest (35.1) Tmean (23) *Urban* (13.7) Pmean (4.7)
Core	Pmean (77.5)	Pmean (56.2) Altitud (11.3) Tmean (8.7) *Urban* (6.4)	Pmean (36.3) Pdriest (14.8) Tmax (11.2) Altitude (5.8) *Urban* (14.3)
Intermediate	Pdriest (8.9) Altitude (11.6) *Agriculture* (8.1) *Protected land* (9) Tmax (24.8) *Heteroagri* (10.8) *Urban* (5.8)	*Agriculture* (23.7) Altitude (26.3) Tmean (18.1) Pdriest (12.1)	*Agriculture* (26) Pdriest (16.7) Pmean (4.5) Tmean (12.1) *Pasture* (11.5) *Urban* (5.6)
Peripheral	*Wetlands* (48.8) Tmean (8.6) *Urban* (10.6) *Protected land* (8.7) Tmincoldestmonth (7.4)	*Protected land* (39.9) *Wetlands* (19.7) Altitude (6.9) *Heteroagri* (12.5) *Pwettestmonth(5.6)*	*Wetlands* (29.1) *Urban* (26.8) *Agriculture* (17.8) *Protected land* (4.5) Altitude (5)

models performed relatively well when evaluated with core sector (1 step), intermediate sector (2 steps) or peripheral sector (3 steps). Lowest β values produced models overfitted to the data and hence the best AUC, sensitivity and kappa results. The ability to predict when 3 steps (national to peripheral) are involved drops quickly as β increases (Fig. 7).

As expected, when a model built for a given sector was used to predict the occurrence in a different sector we obtained weak predictions, and the predictive power of the transferred model was significantly lower than the model built specifically for that particular sector, the differences in all metrics (AUC, sensitivity and kappa) being statistically significant.

When the analysis was carried out to test for differences in both AUC, sensitivity and kappa values among the core, intermediate and peripheral sectors, there was a significant effect of β (Wilks lambda = 0.87 $F_{15,\ 676.74} = 2.26$ $p < 0.004$) and step (Wilks lambda = 0.6 $F_{9,\ 596.42} = 13.59$ $p < 0.0001$) in the values of AUC, sensitivity and kappa. However there were no significant effects of their factorial combination.

Fig. 8 shows the behaviour of AUC, sensitivity of transferred models according to the number of steps involved and the value of β. Models built for each sector (step 0, no transfer) provided the best results with small β values, but incurring the risk of overfitting. One step model transfers (from any given sector to an adjacent sector) performed better with higher β values, while in model transfers involving two steps (i.e. from the core to the peripheral sector o vice versa) the predictive power dropped regardless the value of β. Models built with data from either the core or the intermediate sector and then transferred to the peripheral sector, delivered low quality predictions that adjusted poorly to the actual occurrence data, independently of the β value used (Fig. 8).

The capacity of models to predict Doñana presences is shown in Fig. 9. Core and intermediate models hardly predict them, while peripheral models predict almost the 100% of presences. Only intermediate models worked better with increasing values of β.

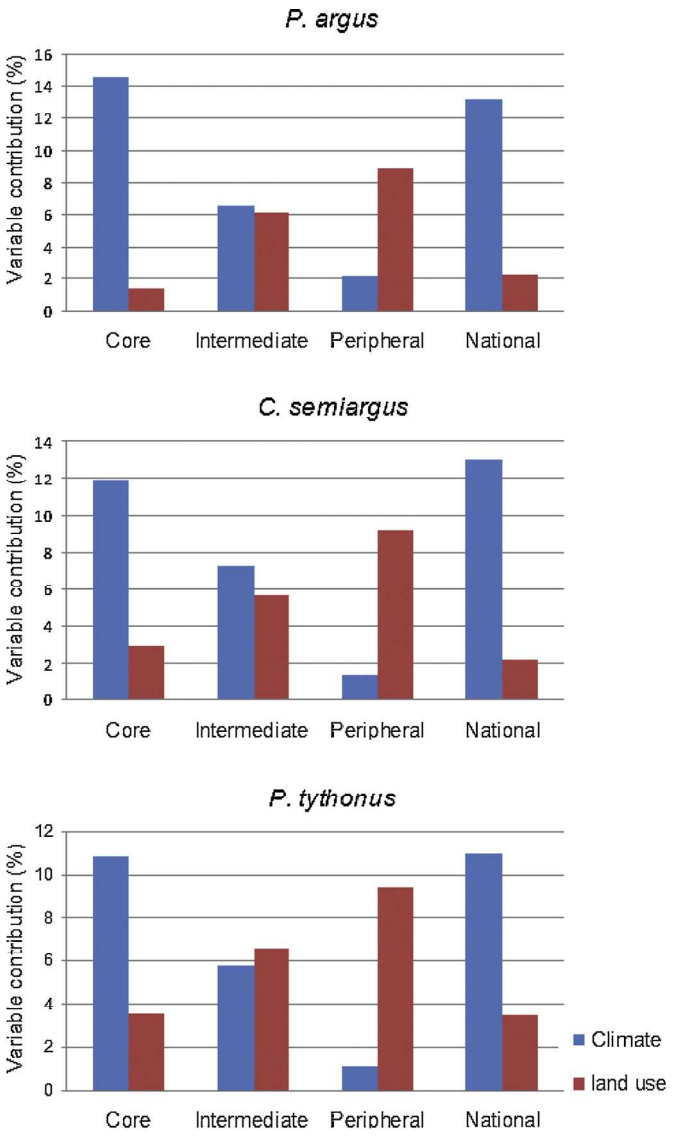

Fig. 5. Percentage of variance explained by the set of climatic variables compared to non-climate ones in each sector of models.

4. Discussion

The main aim of this study was to assess the transferability of MaxEnt distribution models across the distribution area of species. More specifically we tested whether splitting the distribution area in sub-units and modelling the distribution separately for each of these sectors (core, intermediate and peripheral) might lead to better results than using a single global model for the entire area. Our results clearly show that marginal populations of the butterflies *P. argus*, *C. semiargus* and *P. tithonus* respond to climate and habitat differently than core populations from central Spain. The so called national models, developed for the whole training data set, were those that showed better predictive power when they were transferred to the individual sectors. However, after splitting the training data in sectors, transferability decreased as the distance to the target sector where the model was to be applied increased. For example, the model for the core area did not adequately predict occurrences in the peripheral sector, therefore overlooking the large local populations of the three butterfly species in Doñana National Park (Fig. 9). Furthermore, our results pinpoint the fact that peripheral populations near the edge of their ranges can be misrepresented by SDMs. In fact, given that a robust and

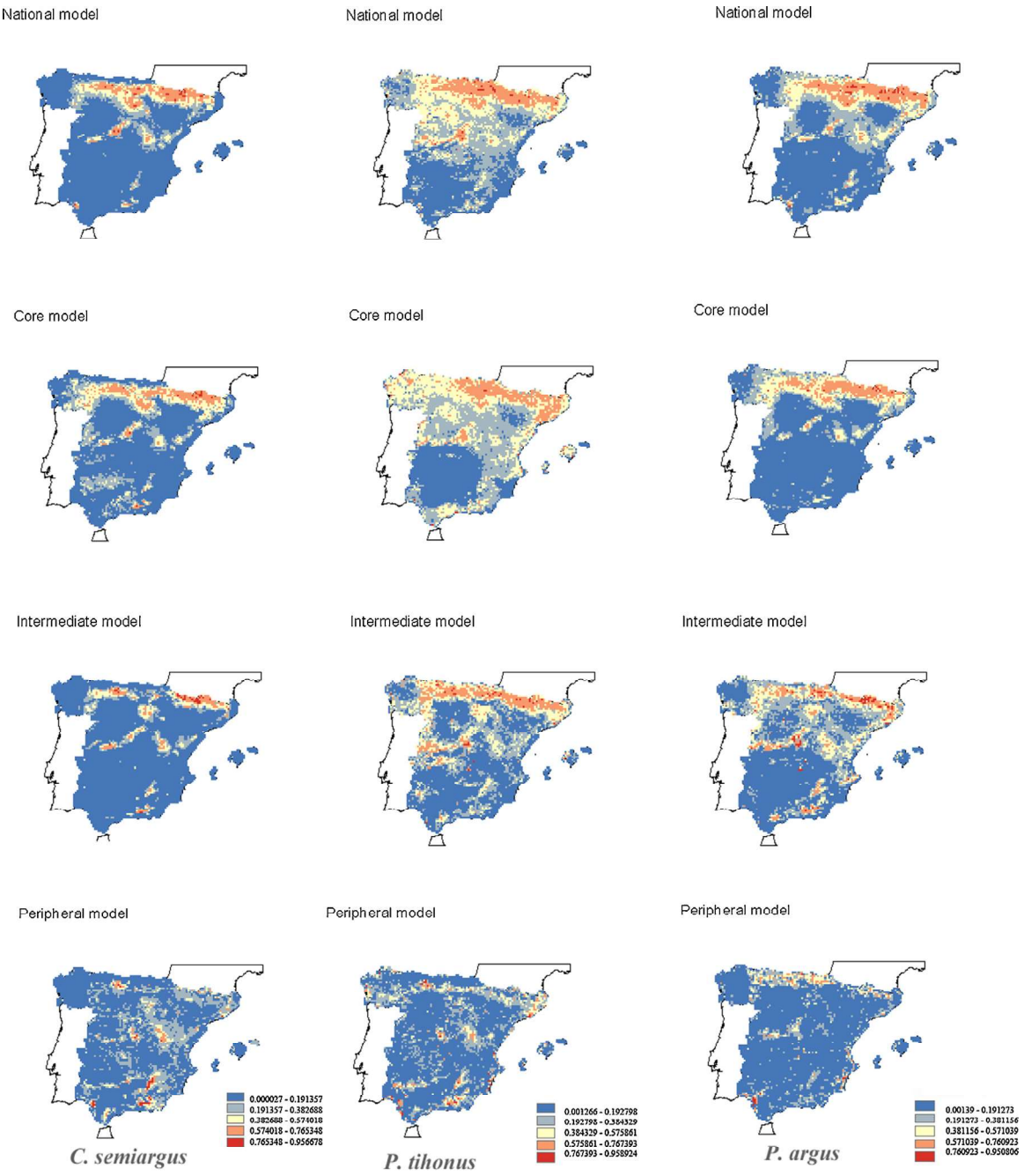

Fig. 6. Potential distribution maps of the target species derived from modelling national (global) data or from data included in each of the three concentric sectors (core, intermediate and peripheral). Legend shows the logistic probability of presence of the species.

representative sample of occurrence data is rarely available, and hence some degree of bias is often inevitable, the probability of finding yet undiscovered local populations should rise towards the margins of the species range.

The values of sensitivity and AUC were high in national models as well as in models for the core, intermediate and especially peripheral sectors when no transfer was involved (with 0 steps), while it was only moderate when one, two or three steps are involved (Figs. 7 and 8). This can be explained by the dependence of this metric on both the species relative occurrence area and data prevalence (Lobo et al., 2007; Wisz et al., 2008). In fact, the peripheral sector included a much smaller sample of data as well as lower prevalence than intermediate and the core sectors. However, Wisz et al. (2008) addressed the possibility of finding different results in the case of naturally impoverished species where the number of occurrence data is low strictly due to natural causes. Occurrences of our three species in the peripheral sector would respond to this pattern, where their restricted distribution in the area is limited by the availability of suitable habitat.

For *P. tithonus* the outermost peripheral sector includes UTM squares almost exclusively located in Doñana, so these squares are more homogeneous in terms of their environmental features than that on the other sectors. However, in the case of periphery sector in *C. semiargus* and *P. argus*, there are just two distant local populations occurring in very different environments. One is Doñana, a flat coastal plain almost at sea level, and the other is the mountain range of Sierra Nevada located at 270 km to the east and where the butterflies occur at 2.400 m (unpublished data). Differences in

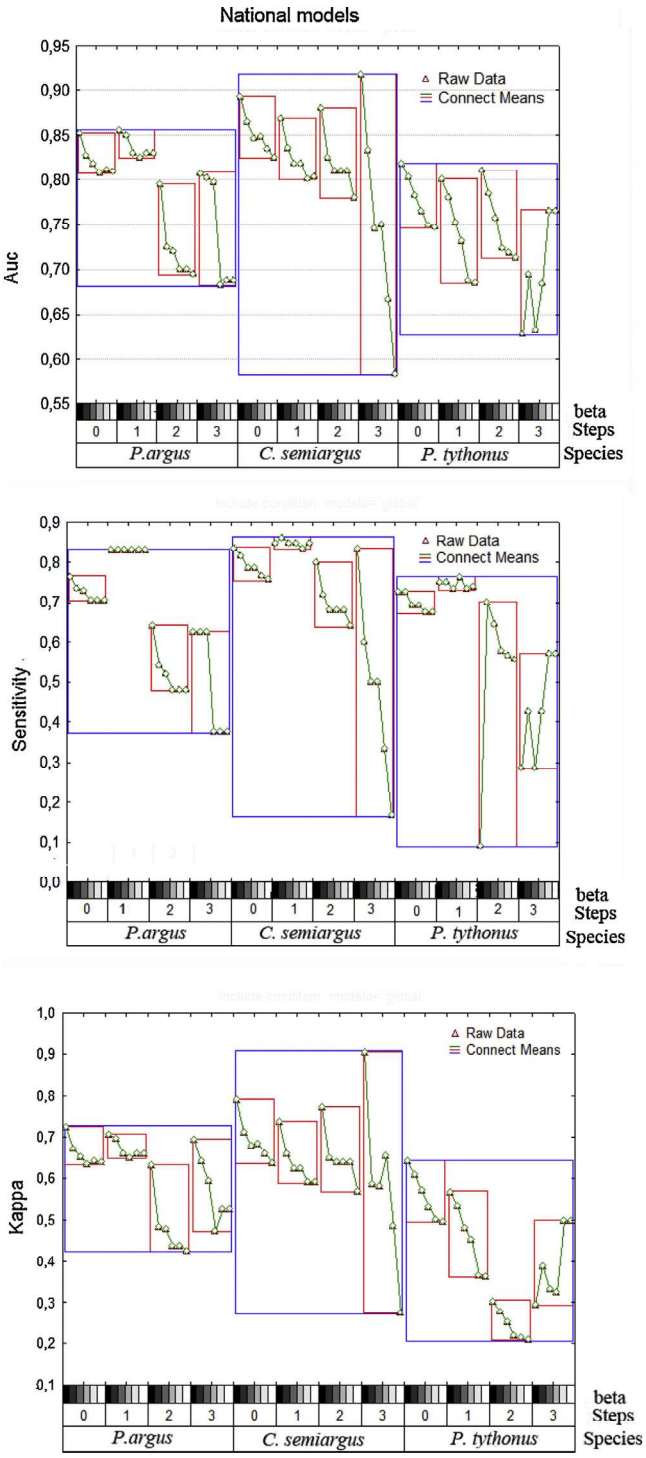

Fig. 7. Behaviour of AUC, kappa and sensitivity values according to β, step and species in national models. Steps refer to discrete distances involved in each model transfer (see Section 2). Gradation in grey represents the range of β values used 0.1, 0.5, 1, 3, 5 and 9, from the lower β value, 0.1, in black to the highest 9 in white.

environmental features between Doñana and Sierra Nevada must be responsible for the low sensitivity value of the models when testing national models on the peripheral sector subsample. In Sierra Nevada the climatic envelope is fairly similar to that found further north in the core distribution area of species. In contrast, there is a huge difference with respect to the climatic envelope of Doñana.

The Kappa index considers both omission and commission errors, thereby resulting in a less biased measure of predictability. In our study the Kappa index yielded low to moderate values, decreasing in the models from the core to the peripheral sector (Fig. 7 and Table 1). This trend may be explained by differences in prevalence among sectors (Fielding and Bell, 1997; Manel et al., 2001; Virkkala et al., 2005).

Higher values of β increase the "smoothness" of species' responses to the environment so that smoother models should be selected when projecting to a new time period or region. In the models presented here certain improvement in transferability were achieved by increasing the values of β, although there was no way of predicting presences in the peripheral sector using the training data from other sectors. In these special cases the capacity of models to predict occurrences out of the training area did not improve significantly after tuning the regularization parameters.

The variability found in the relative contributions of predictor variables to the models pinpoint the importance of examining carefully the set of variables to be used previous to the modelling work. The contribution of non-climatic parameters in the models was highly variable among sectors, and was apparently more important at the edge of species distributions. While for many species MaxEnt can provide robust predictions across large areas using only climatic variables, when modelling distributions in smaller areas like national or regional scales it is clear that models can significantly improve by considering additional environmental variables (Pearson and Dawson, 2003). Although it is widely recognized that climate is the main determinant of the distribution on a continental scale (Thuiller et al., 2004; Rahbek et al., 2007) or at a large spatial resolution (Pearson and Dawson, 2003; Luoto et al., 2007), at finer scales other factors besides climate often play a significant role in determining species occupancy patterns (Brown et al., 1996; Hill et al., 2001; Thuiller et al., 2004; Pearson et al., 2004; Araújo and Pearson, 2005; Virkkala et al., 2005; Araújo and Luoto, 2007; Heikkinen et al., 2007; Luoto et al., 2007). The predictive variables that are used vary, therefore, depending on the scale of research, accepting in general that regional scales are shaped by climatic variables while other variables with more biological influences affect the modelling at local scales (Pearson and Dawson, 2003; Luoto et al., 2007). This has special significance in a large country such as Spain (≈500,000 km^2), with an exceptional variety of climatic, geological and topographical scenarios and with a superimposed ancient human intervention.

For these reasons we include land use variables in addition to climatic variables. Ideally the occurrence data as well as both climatic and environmental data should come from the same period. Although our dataset includes occurrence records spanning from late 19th century to the late 20th century, approximately 75% of the data were gathered in 1970s and 1980s (García-Barros et al., 2004). Land cover data layer (European Environment Agency, 2012) includes data from the 2000–2006 period, therefore some changes in land use and vegetation cover may have certainly happened. However, major changes in the last century have occurred primarily in lowland fertile soils due to intensification and crop shifting, as well as around urban areas where butterflies were already scarce. Mountain areas, protected areas and wetlands have remained more stable.

Other problem to be checked is to take in consideration the environmental novelty in the projected space when transferring models between sectors. In this context, multivariate environmental similarity surfaces, MESS analysis in MaxEnt (Elith et al., 2010) may be useful to identify these variables. Our results show that some environmental variables are outside the range of the training data only when transferred to the periphery sector. More precisely in the peripheral sector temperature is higher and annual rainfall lower than in the intermediate and core sectors. Predictions in these cases should be treated with strong caution (Elith et al., 2010).

Moving away from the central sector, the variables related to land use and especially the presence of wetlands and

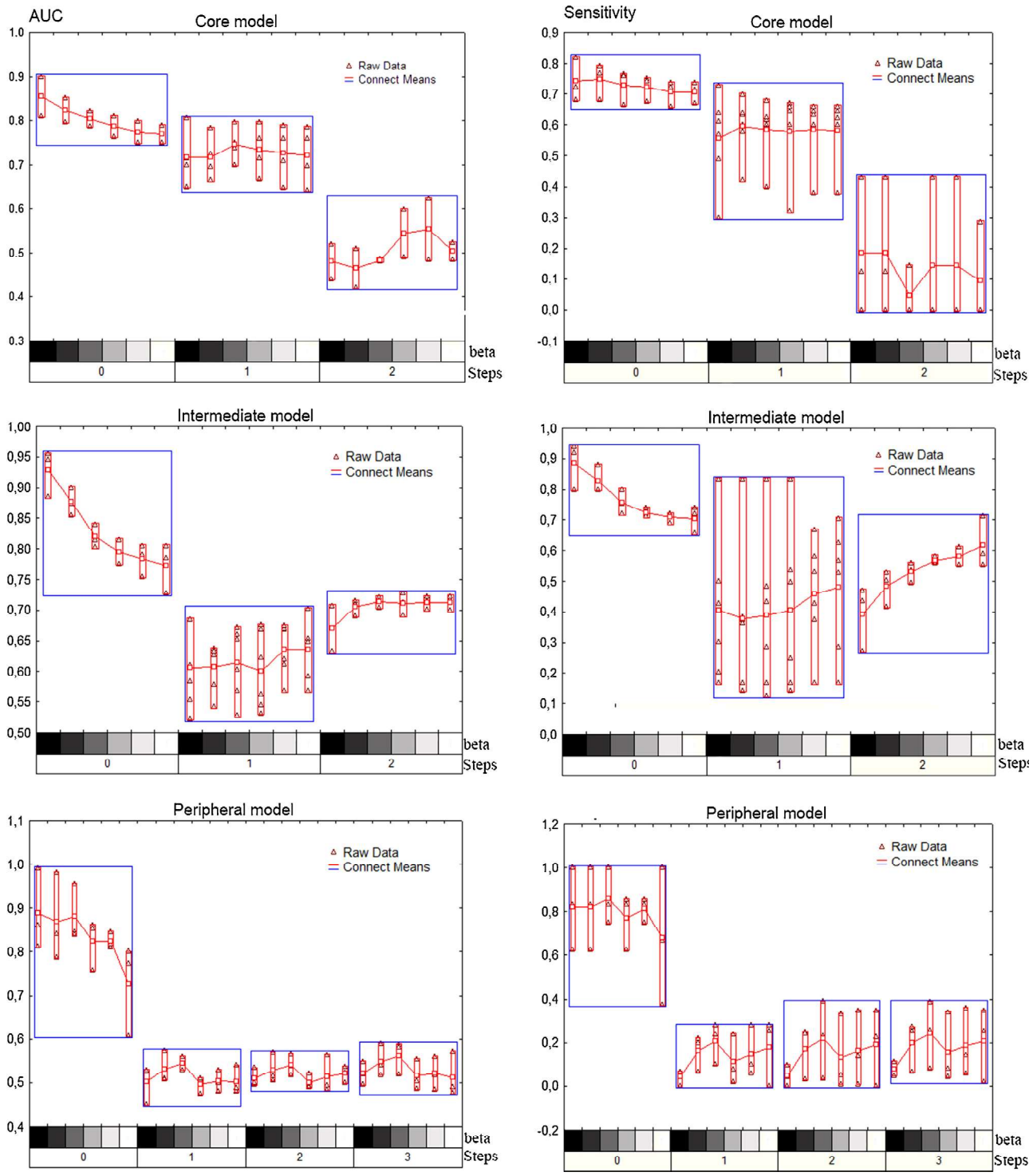

Fig. 8. AUC (right) and sensitivity (left) values of models according to β used and number of steps involved in evaluation. Gradation in grey represents the range of β values used 0.1, 0.5, 1, 3, 5 and 9, from the lower β value, 0.1, in black to the highest 9 in white.

protected areas, gain weight in explaining the distribution of species. Both of these variables are closely linked to the existence of the Doñana national park and its marshes, as well as the Sierra Nevada national park. Species tend to compensate for regional differences in climate conditions by selecting comparable microsites but in different topographical locations (Walter and Walter, 1953). Therefore, species with a wide distribution in the more humid and cooler northern part of Spain tend to present a more fragmented distribution further south. The presence of these species in southern Iberian Peninsula conforms to the law of "relative site constancy" defined by Walter and Walter (1953). Their presence in Sierra Nevada Mountains was fairly predictable, as they can find here climatic conditions similar to the ones they have in their northern distribution. On the contrary, their presence in the Doñana national park, at sea level could be attributed to the existence of a shallow aquifer as evidenced by the occurrence of heathland allowing the existence of species related to wetter climates (García Novo et al., 1977).

There is increasing evidence that bioclimatic models and their predictions need to be applied with caution (Pearson and Dawson,

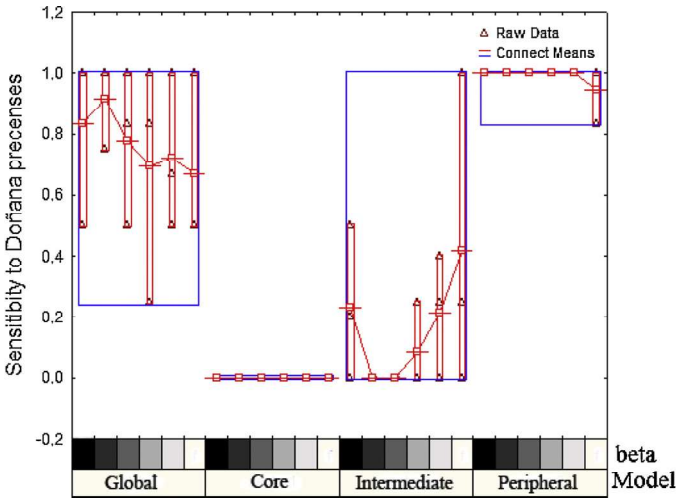

Fig. 9. Sensitivity of models to presences in Doñana National Park. Gradation in grey represents the range of β values used 0.1, 0.5, 1, 3, 5 and 9, from the lower β value 0.1, in black, to the highest 9 in white.

2003; Heikkinen et al., 2007; Mateo et al., 2011). In the case of widespread species the occurrence-environment relationships are weakened by spatial variability in habitat associations and so, it is important to pay attention to the existence of local populations with differences in ecological characteristics so that modelling all these sub-populations together would reduce model accuracy (Stockwell and Peterson, 2002; McPherson and Jetz, 2007). This spatial variability might reflect genetically driven divergence in habitat preferences (local adaptations, Stockwell and Peterson, 2002) or external constraints, with organisms settling for what is best locally. Our sectored approach highlights the fact that certain populations, especially peripheral populations, may be under-represented in the species model. Projecting the national model into the peripheral sector and vice versa proved to be useless, and this suggests that at local scale peripheral populations of some species are also strongly influenced by factors other than climate, including habitat structure, land use and biological interactions, leading to a more fragmented distribution at finer scales (Pearson and Dawson, 2003).

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[CAPITULO 3]

"HABITAT DISTRIBUTION MODELS: ARE MUTUALIST DISTRIBUTIONS GOOD PREDICTORS OF THEIR ASSOCIATES?"

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Resumen

Debido a las limitaciones de tiempo y recursos, los estudios profundos de distribuciones con resoluciones finas en paisajes completos no son viables, y los mapas de distribución deben delinearse utilizando modelos estáticos de hábitat. En los casos en que los ciclos de vida cortos y las limitaciones de tiempo impiden la recopilación de datos detallados de abundancia y distribución para generar modelos de hábitat EN las especies objetivo, una alternativa potencial es utilizar modelos de la abundancia o presencia de especies estrechamente asociadas (por ejemplo, presa, planta huésped, mutualista) para predecir la abundancia u ocurrencia de la especie objetivo.

Presentamos un modelo predictivo para el hábitat de la mariposa *Plebejus argus* en matorrales en un área protegida en el sur de España (Parque Nacional de Doñana), basado en la distribución de su hormiga mutualista *Lasius niger*, datos de campo de hábitat y variables topográficas de un modelo de elevación digital. La frecuencia de hormigueros de *L. niger* fue, con mucho, el principal predictor de la abundancia y presencia-ausencia de *P. argus*. A su vez, la alta frecuencia de *L. niger* se asoció con elevaciones bajas, presencia de brezal y latitudes intermedias dentro del sitio, lo que refleja la profundidad del nivel freático. La frecuencia de *L. niger* predicha por los modelos de 50 cuadrículas de 100 m de calibración se correlacionó significativamente con las frecuencias observadas en 30 cuadrados de evaluación independientes. La abundancia observada de *P. argus* en los mismos sitios de evaluación se correlacionó más estrechamente con la frecuencia pronosticada de *L. niger* solo que con las abundancias predichas de *P. argus* de un modelo que incluye *L. niger* y variables topográficas adicionales. En contraste, la presencia-ausencia de *P. argus* se predijo mejor por los modelos que contenían variables topográficas y por la frecuencia de *L. niger*, aunque también estaba estrechamente relacionada con la frecuencia predicha de *L. niger* solo.

Por lo tanto, nuestro estudio muestra cómo usar distribuciones de especies modeladas para predecir las de sus asociados. También sugerimos que los modelos podrían usarse para evaluar los efectos potenciales de la disminución de la profundidad del nivel freático y los cambios de hábitat resultantes en la distribución y abundancia de *P. argus* en Doñana.

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HABITAT DISTRIBUTION MODELS: ARE MUTUALIST DISTRIBUTIONS GOOD PREDICTORS OF THEIR ASSOCIATES?

DAVID GUTIÉRREZ,[1] PILAR FERNÁNDEZ, ADRIAN S. SEYMOUR, AND DIEGO JORDANO

División de Ecología, Dpto. de Biología Vegetal, Universidad de Córdoba, Colonia San José no. 3, Campus de Rabanales, Córdoba E-14071 Spain

Abstract. Because of time and resource limitations, extensive studies of distributions at fine resolutions over entire landscapes are not viable, and distribution maps must be delineated using static habitat models. In cases where short life cycles and time limitations prevent the collection of detailed abundance and distributional data to generate habitat models for target species, one potential alternative is to use models of the abundance or occurrence of closely associated species (e.g., prey, host plant, mutualist) to predict the abundance or occurrence of the target species. We present a predictive model for the habitat of the butterfly *Plebejus argus* in scrubland in a protected area in southern Spain (Doñana National Park), based on the distribution of its mutualist ant *Lasius niger*, field habitat data, and topographical variables from a digital elevation model. The frequency of nests of *L. niger* was by far the major predictor of *P. argus* abundance and presence–absence. In turn, high *L. niger* frequency was associated with low elevations, presence of heathland vegetation, and intermediate latitudes within the site, reflecting the depth of the water table. *L. niger* frequency predicted by models from 50 calibration 100-m grid squares was significantly correlated with observed frequencies in 30 independent evaluation squares. Observed *P. argus* abundance in the same evaluation sites was more closely correlated with predicted *L. niger* frequency alone than with predicted *P. argus* abundances from a model including *L. niger* and additional topographical variables. In contrast, *P. argus* presence–absence was better predicted by models containing topographic variables as well as *L. niger* frequency, although it was also closely related with predicted *L. niger* frequency alone. Thus, our study shows how to use modeled species distributions to predict those of their associates. We further suggest that the models could be used to assess the potential effects of the declining water table depth and the resulting habitat changes on *P. argus* distribution and abundance in Doñana.

Key words: *ant mutualist; Doñana National Park, Spain; generalized linear model; geographical information system; habitat model;* Halimium halimifolium; *landscape ecology;* Lasius niger; *Lepidoptera;* Plebejus argus; *species distribution.*

INTRODUCTION

Habitat loss, along with habitat alteration and fragmentation, are major threats to biodiversity (Myers 1997). With an increasing rate of species loss, conservation biologists are faced with the task of understanding the dynamics of populations of species in altered and fragmented landscapes (Edwards et al. 1994). In order to evaluate the detrimental effects of landscape changes on particular species, knowledge of their habitat requirements is needed to map the distribution of favorable habitat. However, due to time and resource limitations, habitat mapping of most species is difficult to achieve, especially at a fine resolution over large areas. Usually such large and detailed maps can only be made for those species with very specific and easily located habitat requirements (e.g., Hanski et al. 1994, 1995, Menéndez and Thomas 2000).

However, with the rise of Geographical Information System (GIS) tools, there has been a rapid increase in the use of static habitat models in ecology and conservation biology to predict the distributions of species (e.g., Mladenoff et al. 1995, 1999, Fleishman et al. 2001, Odom et al. 2001, Osborne et al. 2001, Gurnell et al. 2002). The static models statistically relate the geographical distribution of species or communities to the environment without including population or community dynamics (Guisan and Zimmerman 2000). Although there have been some assemblage approaches (Jaberg and Guisan 2001), most of the recently published models were developed for single species and are based on the comparison between the environmental characteristics of a limited number of occupied and unoccupied sites (e.g., Mladenoff et al. 1995, 1999, Odom et al. 2001, Glenz et al. 2001, Gregr and Trites 2001, Parris 2001, Gurnell et al. 2002). If the presence or abundance of a species can be modeled with a reduced number of explanatory habitat variables, the

[1] Present address: Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, Tulipán s/n, Móstoles, Madrid E-28933 Spain. E-mail: d.gutierrez@escet.urjc.es

model can be extended to predict the potential distribution of the species over its entire study region. The final aim of habitat modeling is to obtain a potential distribution map for the species, defined as a cartographic representation of the probability of occurrence or most probable abundance (Guisan and Zimmermann 2000, Austin 2002). Abundance estimates from habitat models can be adjusted upward or downward to account for landscape effects (as matrix and edge effects) using tools such as the Effective Area Model (Sisk et al. 2002; see also Sisk et al. 1997).

Potential distribution maps can be used, for instance, to examine the consequences of a specific environmental change or management regime on the quality and spatial arrangement of the habitat (e.g., Thomas et al. 1999). They may also be used to determine the habitat availability for future recolonization or reintroduction (e.g., Mladenoff et al. 1999, Glenz et al. 2001), or be linked to metapopulation simulation programs that incorporate demographic processes with landscape structure to assess the viability of populations in real landscapes (Akçakaya 2001). In particular, habitat models are extremely useful for defining the borders of patches for species that occupy habitats without clear-cut boundaries, at least from the human perspective.

One major barrier to generating habitat models is the availability of reliable data on presence–absence or abundance of target species. At landscape or geographical scales, collecting these data can be extremely time and effort consuming, especially if the target species has a short-lived stage in which it can be easily observed. One possible approach to overcoming this problem is to formulate models on the basis of key associate species that may be more efficiently sampled, such as specific prey for predators, host plants for herbivores, and mutualists for their obligate associates. This approach may be highly effective if it is easier to model the distributions of the associate species, and if its predicted distribution accurately predicts the distributions of the target species.

In this paper, we test this approach using a butterfly species, *Plebejus argus* (L.) (Lycaenidae), its associate mutualist ant, and its larval host plant, *Halimium halimifolium* (L.) Willk. (Cistaceae), in Doñana National Park, a protected area in southern Spain. In this area, *P. argus* is the most abundant butterfly and its larvae feed on a common shrub species (Rodríguez et al. 1991). *P. argus* has a mutualistic relationship with ants of the genus *Lasius* (Jordano and Thomas 1992). The core distribution of *P. argus* in Doñana is located in a large and relatively homogeneous area of mediterranean scrubland (Rodríguez et al. 1991), where its host plant is extremely abundant, which would appear to provide a continuous habitat for the butterfly (Fig. 1). The biology of this species is relatively well known in Doñana (Rodríguez et al. 1991, Jordano et al. 1992), which provides an excellent opportunity to calibrate and evaluate a fine-grained habitat model based on its associate species and other environmental variables. Potential habitat distribution maps for *P. argus* have previously been generated in a heathland area in southern England with a very different climate, using an extensive GIS database (Thomas et al. 1999). However, the habitat requirements of these northern populations differ greatly from those of southern populations (Jordano et al. 1992).

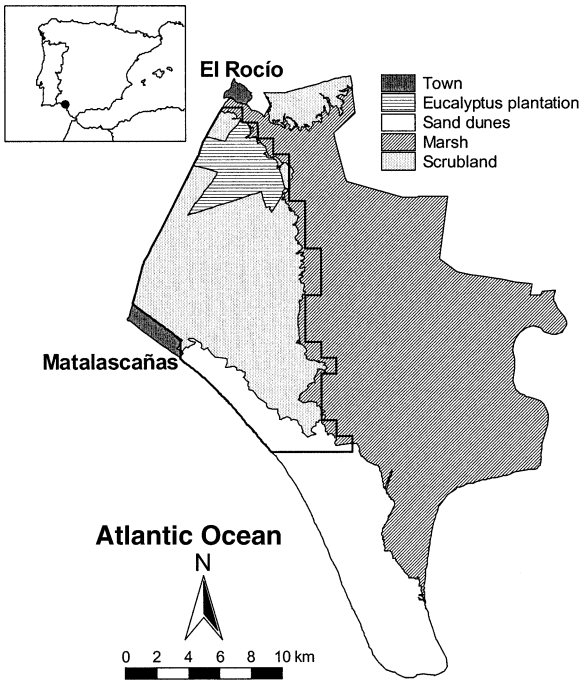

FIG. 1. Map of Doñana National Park, showing the four general types of land cover and main towns. The bold line indicates the study area for which the digital elevation model (DEM) was generated.

Specifically, our study objectives were: (1) to generate models for the abundance and presence–absence of *P. argus* using host plant and mutualist ant abundances and other environmental variables as potential predictors; (2) to derive habitat models for the associate species that significantly accounted for *P. argus* abundance and presence–absence; (3) to evaluate the relative predictive power of habitat models including all significant variables vs. models based only on associate species, as well as models based on all significant variables other than the associate species; and (4), to construct a potential habitat map for *P. argus* using a geographical information system.

METHODS

Study area

The study was carried out in Doñana National Park, on the southwestern coast of Spain (centered on 37°00′ N, 6°25′ W), which covers ~50 720 ha (Fernández-Delgado 1997). The study area is bordered by the At-

PLATE 1. Male *Plebejus argus* basking on the ground. Photo credit: D. Gutiérrez.

lantic Ocean and an urban area to the south and west, by the Guadalquivir and Guadiamar rivers to the east, and by crop lands to the northwest. The landscape in this region is relatively flat and homogeneous, with four subregions dominated by different land cover (Fig. 1). The northernmost region is a highly human-modified landscape consisting of *Eucalyptus calmadulensis* plantations that have been logged during the last few years to promote the regeneration of mediterranean scrubland. Only some small areas of plantations remain. The southernmost and western areas are dominated by a sand dune system bordering the Atlantic Ocean and consisting of a mixture of bare sand areas on dune tops and patches of vegetation (scrubland and *Pinus pinea* woodland) in the troughs between the dunes. The eastern half of the Doñana National Park is covered by an extensive marsh and the central and western areas are mostly occupied by mediterranean scrubland growing on a stable prehistoric sand dune system. In the driest sites, mediterranean scrubland is dominated by species such as *Halimium halimifolium*, *H. commutatum*, *Cistus libanotis*, *Stauracanthus genistoides*, and *Rosmarinus officinalis* (''monte blanco''), whereas in the wettest sites, it is dominated by heathland composed of *Erica scoparia*, *Rubus* sp., and *Ulex minor* (''monte negro'').

The sandy soil in Doñana National Park has an extremely low field capacity, and soil water rapidly drains into the groundwater, or is lost by evapotranspiration after the rainy season. Water table depth is related to elevation and, consequently, soil water availability is markedly limited in the highest sites during late spring and summer (Rodríguez et al. 1994, Fernández-Delgado 1997). Nevertheless, there can be some wet soil areas at high elevations due to some local effects (e.g., drainage to the bottom of slopes).

Study species

The silver-studded blue, *Plebejus argus* (see Plate 1), is a widely distributed butterfly in Europe and Asia (Tolman and Lewington 1997, Kudrna 2002), but its distribution is markedly fragmented (Thomas and Harrison 1992). In Spain, it is widely distributed, except for the lower reaches of the Ebro, Tajo, and Guadiana river basins (Fernández-Rubio 1991). The Doñana populations are the southernmost in Europe and are isolated from the nearest populations in Sierra Nevada in Spain by several hundred kilometers (Rodríguez et al. 1991).

TABLE 1. Environmental and spatial variables used for modeling *Plebejus argus* abundance and *Lasius niger* frequency in fifty 100-m grid squares in Doñana National Park, southern Spain.

Variable	Measurement	Definition
Environmental variables		
Curvature†	DEM	index of concavity–convexity (degrees/100 m)
Eastness†	DEM	index of east–west gradient on a scale from −1 (west-facing) to 1 (east-facing)
Elevation†	DEM	elevation (m)
Northness†	DEM	index of north–south gradient on a scale from −1 (south-facing) to 1 (north-facing)
Slope†	DEM	slope (degrees)
Heathland	field, GIS layer	presence–absence of dense heathland
H. halimifolium density†	field	number of *H. halimifolium* plants in 240 m^2
Spatial variables		
Easting†	DEM	UTM *x*-coordinates at cell center to the nearest m (km)
Northing†	DEM	UTM *y*-coordinates at cell center to the nearest m (km)

Notes: DEM denotes the digital elevation model. *Halimium halimifolium* is the larval host plant for *P. argus*.
† Squared terms were also used in model development. *L. niger* frequency was included as an independent variable for modeling *P. argus* abundance and presence–absence.

The larvae of *P. argus* are relatively polyphagous, potentially feeding on plant species in the Cistaceae, Ericaceae, and Fabaceae families (Fernández-Rubio 1991, Tolman and Lewington 1997). They are strongly myrmecophilous, attended only by species of the genus *Lasius*, specifically *L. niger* and *L. alienus* (Thomas 1985*a*, *b*, Jordano et al. 1992, Jordano and Thomas 1992). The association with ants appears to be more consistent across the range of *P. argus* than the use of particular plant parts and species, and occurs at several spatial scales (Jordano et al. 1992). Nevertheless, *P. argus* larvae appear to be much more host specific at regional scales: in Doñana National Park they feed mostly on leaves of *Halimium halimifolium* (Cistaceae) and are tended by *L. niger* (Rodríguez et al. 1991). *P. argus* eggs are disproportionately laid on the trunk and surrounding litter of *H. halimifolium* plants with a *L. niger* nest at the base. This spatial relationship with ant nests becomes closer for larvae, and even more for pupae, which have only been found attached to plants with *L. niger* nests (Rodríguez et al. 1991, Jordano et al. 1992). In addition, the densities of butterflies in different populations are correlated with *Lasius* ant densities (Jordano et al. [1992]; for other geographical areas, see also Ravenscroft [1990]).

P. argus *and* L. niger *abundances*

During spring 2000, we obtained estimates of the total peak *P. argus* abundance within 50 randomly chosen 100-m UTM grid squares containing *H. halimifolium* plants. Site location was determined with a handheld GPS Garmin 12XL (Garmin Corporation, Olathe, Kansas, USA). To minimize the problems with spatial autocorrelation, selection of 100-m squares was subject to the condition of being located in different 1-km UTM squares. Because the aim of our study was to identify breeding habitat for *P. argus*, the spatial resolution of habitat samples needed to be large enough to encompass sites dominated by population-level processes, i.e., in which birth and death rates largely exceed emigration and immigration rates. As species become more mobile, the correlation between adult distribution and the distribution of breeding habitat at a given resolution is likely to be weaker (Cowley et al. 2000). Because the average dispersal distance for *P. argus* in Doñana is 70 ± 120 m (mean ± 1 SD; median = 28 m; $N = 565$ butterflies; D. Gutiérrez, P. Fernández, A. S. Seymour, and D. Jordano, *unpublished data*), the selection of 100-m grid square resolution appears to be sufficiently large to meet this requirement, but sufficiently small to provide a fine-resolution habitat quality map.

Each 100-m grid square was subdivided into four 50 × 50 m squares in order to ensure an even distribution of samples. Adult counts were carried out along four 50-m transects in each 100-m grid square (one per 50-m square subdivision), totaling 200 m of transect per 100-m square. Transects were walked on sunny days around the approximate peak in the flight season of *P. argus* (from early-to-mid June) according to standard methods in butterfly monitoring schemes (Pollard and Yates 1993). To account for the seasonal variation in butterfly abundance, we walked additional weekly 100-m transects at three independent sites. Counts at each 100-m square were corrected for peak flight date following Thomas (1983). *P. argus* abundance was expressed as the number of adults per 200-m of transect. If no *P. argus* was recorded during the transects, the square was then thoroughly searched to record the presence or absence of butterflies.

During spring 2000, we estimated *L. niger* frequency within the same 50 randomly chosen 100-m UTM grid squares used to obtain *P. argus* abundance. In Doñana, almost all *L. niger* nests are found at the bases of shrubs (*H. halimifolium* and other species) because roots provide suitable sites for nest-building in the sandy soils. Shaking or digging around the plant base produces an explosive response by *L. niger* and other ant species

inhabiting plant roots if there is a nest underneath, which allowed us to categorize *H. halimifolium* plants as (1) with *L. niger*, (2) with another species of ant, or (3) with no ants (Jordano et al. 1992). Following this procedure, we examined all or the first 10 plants encountered along three 10 × 2 m transects approximately evenly distributed within each 50-m square (12 transects per 100-m square). Some sites had low densities of *H. halimifolium*, and consequently the 10 × 2 m transects contained low numbers of plants. To avoid problems with low sample sizes in calculating ant frequencies, in those cases we examined additional plants to obtain a minimum sample of 60 plants per 100-m grid square, or all plants within the square if there were less than 60. *L. niger* frequency was expressed as the proportion of *H. halimifolium* plants with ant nests at the base. We restricted our search to *H. halimifolium* plants (and did not include any other species of shrub) because only *L. niger* nests at the base of this species are important for *P. argus*.

Environmental variables

We described habitat structure on the basis of some variables measured directly in the field and others generated from a GIS database (Table 1). For field variables, data were collected from the same 10 × 2 m transects used for estimating *L. niger* frequencies. Although the literature suggests that density of host plants does not correlate with *P. argus* abundance (Jordano et al. 1992), we estimated the density of *H. halimifolium* plants within each 100-m square by counting the number of shrubs within the 12 transects. This allowed us to explicitly test the predictive power of host plant density vs. *L. niger* density in our data set. It was not possible to access some transects on sites completely covered by dense heathland; in those cases, the density of *H. halimifolium* was assumed to be 0 (*H. halimifolium* is extremely rare in this type of vegetation). Density of *H. halimifolium* plants was expressed as number of plants/240 m^2.

We also recorded in the field the presence–absence of heathland within the 100-m grid square. As stated earlier, heathland vegetation is an indicator of wet habitats.

The topographical characteristics of each 100-m grid square were calculated using a digital elevation model (DEM) of the area shown in Fig. 1 (16 928 cells). Data from contour lines and heights (1-m) of 1:5000 ICONA topographic maps were used as input point theme into ArcView Spatial Analyst to generate a 100-m cell-size DEM (Fig. 2). Subsequently, slope (in degrees), aspect (in degrees), and curvature (in degrees/100 m) for every 100-m cell were derived using ArcView surface analysis functions (Environmental Systems Research Institute 1996; see also Burrough and McDonnell 1998). Curvature is an index of convexity–concavity of a surface at each cell center. A positive curvature indicates that the surface is upwardly convex at that cell, whereas a negative curvature indicates that the surface is upwardly concave, and a value of zero indicates that the surface is flat (Environmental Systems Research Institute 1996). Curvature is an index to define the relative position of a location in a topographic gradient (ridge top, valley, flat surface). For statistical analyses, the circular variable aspect was transformed into a linear north–south gradient (''northness'') and an east–west gradient (''eastness'') using, respectively, the cosine and sine transformations (Guisan et al. 1999). Northness varies on a scale from −1 (south-facing) to 1 (north-facing), and eastness from −1 (west-facing) to 1 (east-facing). Both northness and eastness are environmental variables used to define the relative position of a location in two orthogonal aspect gradients, and they are clearly different from the spatial variables northing and easting that we will explain.

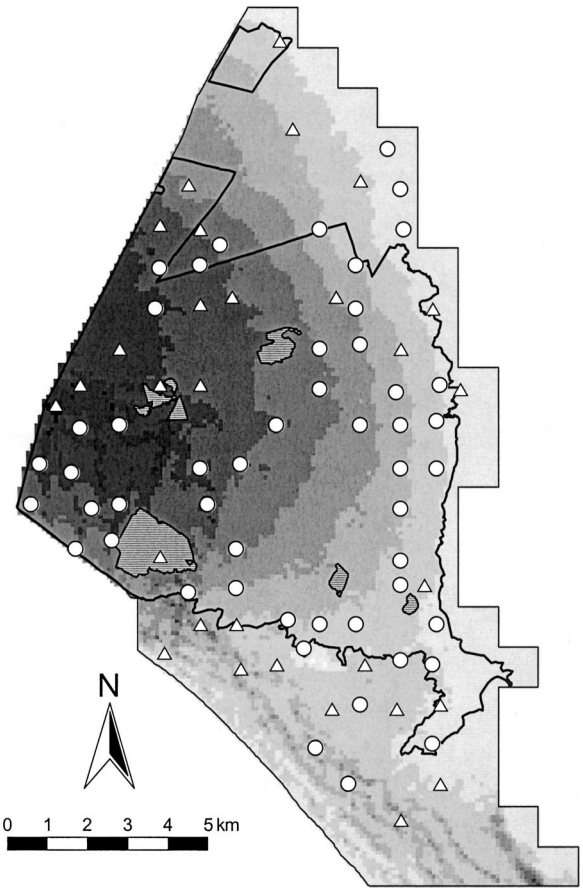

FIG. 2. Topography of the study area within Doñana National Park based on a 100-m grid DEM. Elevation is shaded from low to high (light to dark) using 5-m intervals. The bold line indicates the *Halimium halimifolium* scrubland area for which GLM model predictions of *Lasius niger* frequency were made. Locations of the 50 formulation 100-m squares (circles) and the 30 evaluation 100-m squares (triangles) are also shown. The horizontally striped areas are dense *Pinus pinea* plantations.

Model formulation

We used generalized linear models, GLM (McCullagh and Nelder 1989) using S-PLUS software (MathSoft 1999) to model *P. argus* abundance, *P. argus* presence–absence, and *L. niger* frequency using the independent variables measured in the habitat survey (Table 1). Because the distribution of the response variable *P. argus* abundance is actually a count from 0 to infinity, we applied a quasi-likelihood estimation of the regression coefficients using a log-link, and setting the variance equal to the mean (Venables and Ripley 1997, MathSoft 1999). The quasi-likelihood estimation allows regression coefficients to be estimated without fully knowing the error distribution of the response variable. This method gives an estimate of the dispersion parameter of the variance function, which otherwise is set to equal one using the standard Poisson GLM models (the default value; MathSoft 1999). For model building, we included the environmental variables in Table 1. Because linear relationships may be insufficient to characterize the response of a species to environmental variables, we incorporated squared terms into the model for continuous variables (Table 1) to allow for potential unimodal responses of *P. argus* abundance (Jongman et al. 1995). Model selection was carried out using a forward-selection procedure, starting with a null model (intercept only) to which individual terms were added. At each step, one term is selected on the basis of the magnitude of the C_p statistic estimated for each term until no additional terms improved the model (Venables and Ripley 1997). The change of deviance of the model by the inclusion of a term was tested against an *F* distribution (MathSoft 1999). The fit of the final model was characterized by D^2, the equivalent to r^2 in least squares models (Guisan and Zimmermann 2000).

We modeled *P. argus* presence–absence and *L. niger* frequency using the same procedure as for *P. argus* abundance. However, given that the distributions of the response variables are actually a binary response (0, 1; *P. argus*) and a probability ranging from 0 to 1 (*L. niger*), we applied a quasi-likelihood estimation of the regression coefficients using a logit-link and setting the variance to [mean(1 − mean)] (Venables and Ripley 1997, MathSoft 1999).

In order to evaluate the importance of *L. niger* frequency in modeling *P. argus* abundance and presence–absence, we generated three different models: (1) a model including all potential significant variables; (2) a model including *L. niger* frequency only; and (3) a model including all potential significant variables other than *L. niger* frequency.

Spatial structure of data

Recently, the importance of spatial structure and autocorrelation when building habitat models has been emphasized (Lennon 1999). One unattended, but important, ecological problem is that spatial autocorrelation results in a bias toward generating habitat models that include strongly spatially autocorrelated environmental variables, even if these variables are not actually significant (Lennon 2000). Lennon (1999) has suggested that the autocorrelation of the response variable is the main problem; if this variable is not spatially autocorrelated, the spatial structure in the explanatory variables is unimportant.

To model the spatial variation of *P. argus* abundance, *P. argus* presence–absence, and *L. niger* frequency, we include the UTM *x*- (easting) and *y*-coordinates (northing) of the sample grid cells as independent variables in the GLM model (see *Methods: Model formulation*). These variables simply locate a sample in geographical space and they are different from the environmental variables eastness and northness, as we have explained. Including spatial coordinates when modeling is called trend surface analysis, and this method is the simplest way to account for gradual, long-range spatial variation in the data (Legendre 1993, Jongman et al. 1995). However, given that there may be more complex spatial structures in the data, we also generated all-directional correlograms (Legendre and Fortin 1989). Characteristic shapes of correlograms are associated with particular types of spatial structures. To generate each correlogram, we plotted values of Moran's *I* coefficient against Euclidean distance classes between sampling grid cells (Legendre and Fortin 1989). We divided distances into classes by forming equal distance intervals. Given that spatial autocorrelation analysis should not be performed with a small number of localities (fewer than ~30; Legendre and Fortin 1989), we divided our data set into five distance classes. Thus, the smallest sample size was 26 (for the class including the longest distances). We tested significance for each Moran's coefficient using 4999 Monte carlo permutations, the realistic minimum to estimate a significance level of ~0.01 (Manly 1994). Before examining each individual Moran's coefficient value in each correlogram, we first tested for its overall significance. This was performed by checking whether there was at least one significant value after Bonferroni correction (the critical *P* value divided by the number of individual tests; for details, see Legendre and Fortin [1989]). Moran's *I* coefficients and significance tests were performed using Rookcase (Sawada 1999).

Model evaluation

To evaluate the predictive power of the habitat models, we randomly chose 30 UTM 100-m grid squares that were located in 30 different UTM 1-km squares, and different from those 50 squares used for calibrating the model. Using the same sampling strategy as for the model calibration samples, we performed the field survey in spring 2001 and measured the abundance and presence–absence of *P. argus*, the frequency of *L. niger*, and the same habitat characteristics as at the cal-

ibration sites. The topographical characteristics of each grid cell were derived from the DEM. For *P. argus* abundance and *L. niger* frequency, which are continuous variables, the evaluation procedure of GLM models consisted of calculating Spearman rank correlation coefficients for estimating the fit between observed and predicted values (Guisan and Zimmermann 2000). To test for consistent bias of predicted values, we used Wilcoxon signed-ranks tests (Pasinelli 2000). For *P. argus* abundance, we also tested the correlation between that variable and the predicted *L. niger* frequency. This test allowed us to estimate whether predicted mutualist frequency alone is an indicator of abundance of its associate species, *P. argus*.

For evaluating the significance of predictions of *P. argus* presence–absence, we performed a logistic regression between observed binary values and predicted probabilities of presence derived by the GLM models. We also derived a discrimination index from the area under a relative operating characteristic (ROC) curve. ROC analysis is independent of both species prevalence and decision threshold effects (Pearce and Ferrier 2000, Manel et al. 2001). We used the nonparametric Mann-Whitney technique to calculate the ROC index of discrimination ability because it makes no distributional assumptions. This index deals with the ability of a model to distinguish between occupied and unoccupied sites, and it is an estimate of the probability that the model will predict a higher likelihood of occurrence for a positive observation than for a negative observation if both are selected at random (Pearce and Ferrier 2000). To test whether *L. niger* frequency alone significantly correlates with *P. argus* presence, we also performed a logistic regression including both variables. Analyses were performed using SPSS (Norusis 1993).

Because winter and spring 1999–2000 were relatively dry (262.1 mm/m^2 in December 1999–June 2000), and winter and spring 2000–2001 were extremely wet (605.5 mm/m^2 in December 2000–June 2001), the inter-year variability in *P. argus* abundance and *L. niger* frequency was also assessed in 2001 by examining 10 100-m squares that were also studied in 2000 (the ''repeated squares,'' henceforth). Based on the data recorded in 2000, we chose the 10 repeated squares to approximately cover the whole range of values of *P. argus* abundance and *L. niger* frequency. The inter-year variability in the ranking of both variables was assessed using the Spearman rank correlation coefficient.

Model predictions and mapping

Once *P. argus* abundance and presence–absence and *L. niger* frequency responses were derived by GLM modeling, we extended the significant models to all 100-m squares over a 92.36 km^2 area dominated by *H. halimifolium* scrubland (Figs. 2 and 3). The host plant, *H. halimifolium*, has a patchy and sparse distribution in the sand dunes and the *E. calmadulensis* and dense *P. pinea* plantation areas (Fig. 1) and, consequently, its distribution cannot be inferred from aerial photographs at 100-m grid cell resolution (i.e., those areas were excluded from predictions). The presence of heathland at each 100-m grid square was derived from a GIS vectorial layer using Arc View Spatial Analyst functions (Environmental Systems Research Institute 1996). The heathland GIS layer was generated by digitizing the vegetation patches on scanned aerial photographs corrected for distortion and UTM geo-referenced (Fig. 3). To evaluate the accuracy of the heathland GIS map, we set out a contingency table of observed (from the field) and interpreted (from aerial photographs) presence–absence of heathland at each 100-m grid square used for formulating and evaluating the habitat model (i.e., 80 squares). We used the chi-square test with continuity correction and the kappa statistic to test the agreement between the observed proportion of correctly classified 100-m grid squares and the proportion expected by chance; kappa = $(P_{obs} - P_{exp})/(1 - P_{exp})$, where P_{obs} is the observed proportion correctly classified and P_{exp} is the expected proportion

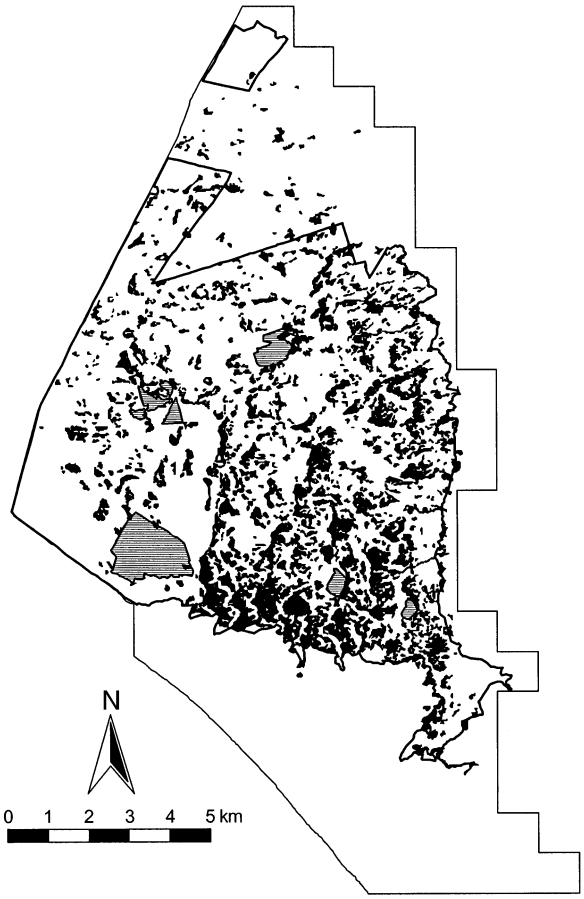

FIG. 3. Distribution of heathland patches (black areas) in the study area shown in Fig. 1 within the Doñana National Park. The horizontally striped areas are dense *Pinus pinea* plantations.

TABLE 2. Model parameters and fit for the GLM models for estimating the effects of (a) all environmental variables in Table 1, (b) *L. niger* frequency only, and (c) all variables other than *L. niger* frequency on *P. argus* abundance.

Variable	df	Coefficient (1 SE)	*t*	*P* for *t* test	Residual deviance	*P* for *F* test
Null model	49				2126.095	
a) All environmental variables						
Intercept		−152.21 (51.58)	−2.951	0.005		
L. niger frequency	1	4.546 (0.588)	7.729	<0.001	1036.050	<0.001
Curvature	1	30.373 (5.755)	5.278	<0.001	714.855	<0.001
Easting	1	0.211 (0.071)	2.971	0.005	588.217	0.004
Dispersion parameter = 13.47						
b) *L. niger* frequency						
Intercept		1.612 (0.308)	5.225	<0.001		
L. niger frequency	1	3.998 (0.562)	7.108	<0.001	1036.050	<0.001
Dispersion parameter = 22.57						
c) All variables except *L. niger* frequency						
No significant variables entered						

Notes: The models were derived by quasi-likelihood estimation using a log-link and setting the variance term to the mean. Significant terms were included using stepwise addition of terms to the null model, based on the magnitude of the C_p statistic at each step and *F* tests ($P < 0.05$). Terms are shown in decreasing order of their contribution to reduce the C_p statistic at each step. Definitions of the variables are in Table 1.

by chance (Wyatt 2000). We also excluded the 100-m grid squares only occupied by heathland from the predictions, because in those cases the host plant was absent.

GLM models are easily implemented in GIS by building a single formula in which each coefficient multiplies its related predictor variable. In GLMs, the results of the calculations are obtained on the scale of the linear predictor of the model, so it was necessary to apply the inverse log and inverse logistic transformations to obtain values, respectively, of *P. argus* abundance, and *P. argus* presence–absence and *L. niger* frequency (Guisan and Zimmermann 2000). In ArcView, formulas are readily implemented in the map calculator functions by using grid themes of the corresponding predictor variables (Environmental Systems Research Institute 1996).

RESULTS

Habitat survey

In 2000, we recorded a total 708 *Plebejus argus* adults in the transects in the 50 calibration squares. Corrected peak abundances ranged from 0 to 169 individuals per 200-m transect. *P. argus* was present in 26 (52%) of the 50 calibration squares. We measured and checked for ants in a total of 4002 *Halimium halimifolium* plants in the 50 calibration 100-m squares, giving a *Lasius niger* frequency ranging from 0 to 0.848. The elevation range of the 50 squares (1.7–36.1 m) covered a high percentage of the elevation range contained in the DEM of the study area (0.1–43.8 m), and 21 squares (42%) contained some heathland. Pairwise distances between sites averaged 6322 ± 3039 m (mean ± 1 SE), $N = 1225$, range = 600–15 931 m).

Model formulation

The results of the GLM models of *P. argus* abundance are given in Table 2. The model with all potential variables accounted for ~72% of deviance, and included *L. niger* frequency as the most important predictor of *P. argus* abundance (Table 2a). The remaining deviance accounted for curvature and a spatial component, easting. These results indicate that *P. argus* abundance increases with increasing *L. niger* frequency, as the site becomes more upwardly convex and toward the east. As could be expected, the model with only *L. niger* frequency was also significant and accounted for 51% of deviance (Table 2b). However, when *L. niger* frequency was excluded from the potential explanatory variables, no variables significantly entered during the stepwise modeling.

The results of the GLM models of *P. argus* presence–absence are shown in Table 3. The model with all potential variables explained ~46% of deviance (Table 3a). *P. argus* was more likely to occur in sites with higher *L. niger* frequency, the presence of heathland, and steeper slopes. The model with *L. niger* frequency only was also significant and accounted for 30% of deviance (Table 3b). Finally, when *L. niger* frequency was excluded from the potential explanatory variables, four variables significantly entered during the stepwise modeling process: presence of heathland, northing, and its quadratic term, and easting, all together accounting for 53% of deviance (Table 3c).

The results of the GLM model of *L. niger* frequency are given in Table 4. The logistic model explained 55% of deviance, and included four significant terms: a quadratic negative term for elevation, a positive term for the presence of heathland, and both positive and quadratic negative terms for northing. These results indi-

TABLE 3. Model parameters and fit for the GLM models for estimating the effects of (a) all environmental variables in Table 1, (b) *L. niger* frequency only, and (c) all variables other than *L. niger* frequency on *P. argus* presence–absence.

Variable	df	Coefficient (1 SE)	*t*	*P* for *t* test	Residual deviance	*P* for *F* test
Null model	49				69.235	
a) All environmental variables						
Intercept		−2.546 (0.703)	−3.621	<0.001		
L. niger frequency	1	12.184 (3.579)	3.404	0.001	48.247	<0.001
Heathland (presence)	1	2.417 (0.792)	3.054	0.004	41.610	0.005
(Slope)^2	1	0.775 (0.500)	1.723	0.092	37.713	0.029
Dispersion parameter = 0.77						
b) *L. niger* frequency						
Intercept		−1.113 (0.432)	−2.575	0.013		
L. niger frequency	1	10.712 (3.480)	3.079	0.003	48.247	<0.001
Dispersion parameter = 0.97						
c) All variables except *L. niger* frequency						
Intercept		−2 320 218 (937 305)	−2.475	0.017		
Heathland	1	3.057 (1.203)	2.541	0.014	56.374	<0.001
(Northing)^2	1	−0.138 (0.056)	−2.476	0.017	47.684	0.004
Northing	1	1132.18 (457.34)	2.476	0.017	40.484	0.008
Easting	1	0.412 (0.160)	2.570	0.013	32.272	0.005
Dispersion parameter = 0.95						

Notes: The models were derived by quasi-likelihood estimation using a logistic-link and setting the variance term to [mean(1 − mean)]. Significant terms were included using stepwise addition of terms to the null model, based on the magnitude of the C_p statistic at each step and *F* tests ($P < 0.05$). Terms are shown in decreasing order of their contribution to reduce the C_p statistic at each step. Definitions of the variables are in Table 1.

cate that *L. niger* frequency increased curvilinearly with decreasing elevation and with the presence of heathland in the grid square. In addition, there is a Gaussian response to the spatial north–south gradient.

Spatial structure of data

The Bonferroni-corrected critical *P* value was 0.01 for the three correlograms. The correlogram for the *P. argus* abundance data was globally significant at the critical 0.05 level because the first value of spatial autocorrelation was positive and significant at the corrected *P* value, corresponding to the sampling grid squares in the distance class 0–3.2 km (Fig. 4). The correlogram for *P. argus* presence–absence was also U-shaped and highly significant, with all values other than the distance range 12.8–16 km being significant after the Bonferroni correction (Fig. 4). The correlogram for *L. niger* frequency was also globally significant. In this case, the first value of Moran's *I* was positive and significant at the corrected *P* value, and the third value was negative (distance classes 6.4–9.6 km). The remaining values were not significant.

To test whether the spatial structure of the data still remained after accounting for environmental variation, we generated correlograms using the residuals of *P. argus* abundance and presence–absence and *L. niger* frequency from the GLM models shown in Tables 2a, 3a, and 4, excluding the spatial components. We found no significant and close-to-zero Moran's *I* coefficients for the *P. argus* abundance and presence–absence residuals, indicating that there was no apparent spatial structure in the data set once the environmental variation was taken out (Fig. 4). The correlogram for *L. niger* frequency residuals was still globally significant

TABLE 4. Model parameters and fit for the GLM model for estimating the effects of the environmental variables in Table 1 on *L. niger* frequency.

Variable	df	Coefficient (1 SE)	*t*	*P* for *t* test	Residual deviance	*P* for *F* test
Null model	49				17.307	
Intercept		−634 889 (290 152)	−2.188	0.034		
(Elevation)^2	1	−0.0028 (0.0008)	−3.300	0.002	12.953	<0.001
Heathland (presence)	1	0.9911 (0.4094)	2.421	0.020	10.309	<0.001
(Northing)^2	1	−0.0378 (0.0173)	−2.190	0.034	8.891	0.009
Northing	1	309.880 (141.567)	2.189	0.034	7.790	0.021
Dispersion parameter = 0.19						

Notes: The model was derived by quasi-likelihood estimation using a logit-link and setting the variance term to [mean(1 − mean)]. Significant terms were included using stepwise addition of terms to the null model, based on the magnitude of the C_p statistic at each step and *F* test. Terms are shown in decreasing order of their contribution to reduce the C_p statistic at each step. Definitions of the variables are in Table 1.

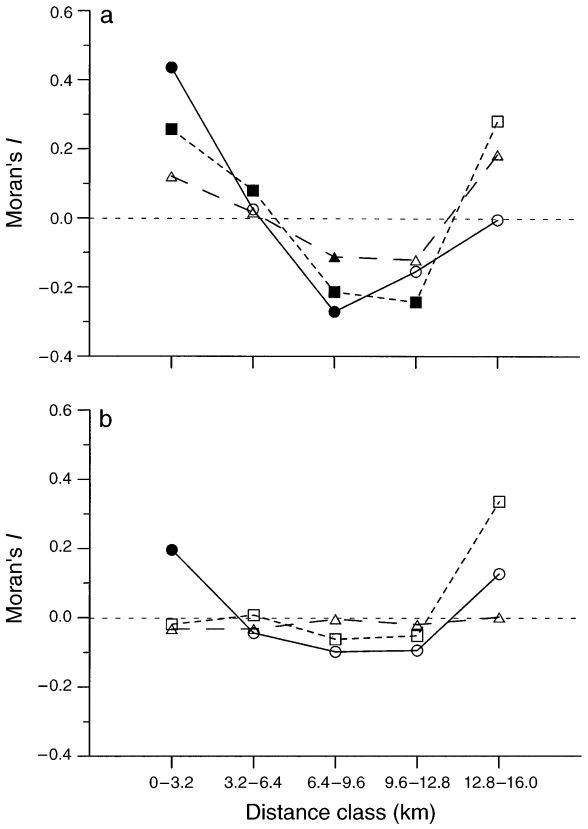

FIG. 4. All-directional spatial correlograms using Moran's *I* of *Plebejus argus* abundance (triangles, dashed line), *P. argus* presence–absence (squares, dotted line), and *L. niger* frequency (circles, solid line) data on the 50 calibration 100-m squares for (a) the raw data, and (b) the residuals of the GLM models in Tables 2a, 3a, and 4 once the environmental variables were factored out. Environmental variables: *L. niger* frequency and curvature for *P. argus* abundance; *L. niger*, heathland, and (slope)^2 for *P. argus* presence–absence; and (elevation)^2 and heathland for *L. niger*. The width of each distance class is 3.2 km. Solid symbols represent significant values after applying the Bonferroni correction to test the overall significance of the correlogram (critical *P* value = 0.01 for five distance classes); blank symbols are nonsignificant values. Sample sizes from left to right for all correlograms are 224, 417, 403, 155, and 26 locality pairs. The fine dashed line represents $I = 0$.

because the first value of Moran's *I* coefficient was significant at the corrected *P* value (Fig. 4).

Model evaluation

In 2001, we recorded 115 *P. argus* adults in the transects in the 30 evaluation squares and looked for ant nests in a total of 2446 *H. halimifolium* plants. *P. argus* occured in 18 (60%) out of the 30 evaluation squares. The elevational range of evaluation sites (2.0–37.8 m) covered a large percentage of the elevational range of the entire study area, and 12 (40%) grid squares contained heathland.

In the repeated squares, *P. argus* abundance in 2000 was highly correlated with that in 2001 ($r_S = 0.895$, $N = 10$, $P < 0.001$), suggesting that abundance ranking across sites was relatively constant between both years. We found a similar trend for *L. niger* frequency in 2000 and 2001 ($r_S = 0.855$, $N = 10$, $P = 0.002$).

The results of the evaluation tests for *L. niger* frequency and *P. argus* abundance and presence–absence are included in Table 5. There was a significantly positive relationship between the predicted values of *L. niger* frequency on the basis of the GLM model in Table 4 and the observed values in the 30 evaluation squares in 2001. We found no significant difference in paired comparisons between observed and predicted *L. niger* densities (Wilcoxon signed-ranks test: $Z = -0.134$, $N = 30$, $P = 0.894$).

Because *L. niger* was the major predictor of *P. argus* abundance and presence–absence in the GLM models shown in Tables 2a, 2b, 3a, and 3b, we calculated the predicted values using both observed and predicted values of *L. niger* frequency; therefore, we tested the predictive ability of the models regarding both possibilities. For the model including all significant variables (from Table 2a), we found a significant positive correlation between observed and predicted values of *P. argus* abundance when observed *L. niger* frequency was used in the calculations. However, this relationship lost significance when we used predicted *L. niger* frequency. Wilcoxon signed-ranks tests showed significant differences in paired comparisons between observed and predicted *P. argus* abundances when using observed *L. niger* in the calculations ($Z = -2.067$, $N = 30$, $P = 0.039$), but not when using predicted *L. niger* ($Z = -1.820$, $N = 30$, $P = 0.069$). For the model with *L. niger* frequency only (from Table 2b), observed and predicted values of *P. argus* abundance were highly positively correlated both when observed and predicted *L. niger* were used in the calculations. However, predicted values of *P. argus* abundance from the models tended to be significantly higher than observed values in both cases ($Z = -4.460$, $N = 30$, $P < 0.001$; and $Z = -3.898$, $N = 30$, $P < 0.001$, when using observed and predicted *L. niger* frequency in the calculations, respectively). Because no significant model was found when excluding *L. niger* frequency during the variable selection process (Table 2c), no evaluation test was performed in this case. Finally, observed *P. argus* abundance and predicted *L. niger* frequency were significantly correlated.

For the model including all significant variables (from Table 3a), the logistic regressions of observed *P. argus* presence–absence against predicted *P. argus* probability of presence were highly significant when using both observed and predicted *L. niger* frequencies in the calculations. The discrimination capacities of these models tested by ROC methods with the Mann-Whitney statistic were 0.866 ($P = 0.001$) and 0.870 ($P = 0.001$) when using, respectively, observed and predicted *L. niger* frequencies in the calculations. This result indicates that, in both cases, the models can cor-

TABLE 5. Results of evaluation of GLM models included in Table 2 (*P. argus* abundance), Table 3 (*P. argus* presence–absence), and Table 4 (*L. niger* frequency) using field data from 30 independent 100-m grid squares sampled in 2001.

Field-observed variables	Model-predicted variables	Statistic†	*P*
L. niger frequency	*L. niger* frequency	0.696	<0.001
P. argus abundance	*P. argus* abundance (all variables, based on observed *L. niger* frequency)	0.435	0.016
	P. argus abundance (all variables, based on predicted *L. niger* frequency)	0.290	0.121
	P. argus abundance (model with *L. niger* frequency only, based on observed *L. niger* frequency)	0.612	<0.001
	P. argus abundance (model with *L. niger* frequency only, based on predicted *L. niger* frequency)	0.512	0.004
	P. argus abundance (all variables other than *L. niger* frequency)	NA	
	L. niger frequency	0.512	0.004
P. argus presence–absence	*P. argus* probability of presence (all variables, based on observed *L. niger* frequency)	0.394 (+)	<0.001
	P. argus probability of presence (all variables, based on predicted *L. niger* frequency)	0.448 (+)	<0.001
	P. argus probability of presence (model with *L. niger* frequency only, based on observed *L. niger* frequency)	0.185 (+)	0.016
	P. argus probability of presence (model with *L. niger* frequency only, based on predicted *L. niger* frequency)	0.148 (+)	0.022
	P. argus probability of presence (all variables other than *L. niger* frequency)	0.377 (+)	<0.001
	L. niger frequency	0.176 (+)	0.011

† The statistical significance of the relationship between observed and predicted variables was tested by Spearman rank correlation coefficients (r_S) for *P. argus* abundance and *L. niger* frequency) and by logistic regression (D^2 and sign) for *P. argus* presence–absence). ''NA'' indicates not applicable.

rectly discriminate between occupied and unoccupied sites ~87% of the time. For the model including *L. niger* frequency only (from Table 3b), we found a significant relationship between observed presence–absence and predicted probability of *P. argus* presence, both using observed and predicted *L. niger* in the calculations. The discrimination capacity (Mann-Whitney statistic) was also high in both cases (0.808, $P = 0.005$ and 0.806, $P = 0.005$, using predicted and observed *L. niger* frequencies in the calculations, respectively). For the model including all significant variables other than *L. niger* frequency (from Table 3c), there also was a significant relationship between observed presence–absence and predicted probability of *P. argus* presence. The discrimination capacity was highly significant (Mann-Whitney statistic = 0.898, $P < 0.001$). Finally, logistic regression analysis indicated that observed *P. argus* presence was positively related to predicted *L. niger* frequency.

Model predictions and mapping

The heathland patches digitized from aerial photographs are shown in Fig. 3. The percentage of correct classifications of 100-m squares was 92.5%, giving kappa = 0.844. The association between observed and photointerpreted presence–absence of heathland was highly significant ($\chi^2 = 53.653$; df = 1; $P < 0.001$).

We used the final GLM model in Table 4 to create a map of predicted *L. niger* frequency in the area dominated by *H. halimifolium* scrubland. Continuous *L. niger* frequency was reclassified into four categories for mapping (Fig. 5). The map shows that there is a core area with high predicted *L. niger* frequency in the southern and eastern portions of the scrubland area, corresponding to sites with relatively low elevation and large patches of heathland (Figs. 2 and 3). In contrast, the northern and western parts contain 100-m grid squares with low *L. niger* frequency surrounded by extremely low *L. niger* frequencies, which generates a patchy pattern. There was substantial heterogeneity in the percentage of 100-m squares predicted to contain different *L. niger* frequencies (Fig. 6). Half of the squares had predicted *L. niger* frequency equal to, or smaller than 0.1, whereas only ~10% of squares contained predicted *L. niger* frequency higher than 0.5.

The potential distribution map of *P. argus* based on GLM model in Table 3a shows a large suitable (probability of occurrence > 0.75) zone for the species in the south and east of the scrubland area, overlapping with an area with high *L. niger* frequency and large patches of heathland (Fig. 7). On the contrary, most of the landscape in the west is predicted as unsuitable (probability of occurrence < 0.5, using a cutoff of 0.5), apart from some areas next to heathland patches. Of the 100-m grid squares, 42% predicted *P. argus* probabilities of occurrence <0.5 (Fig. 8).

DISCUSSION

Spatial structure of data

Our results revealed that there was some spatial structure in the raw *Plebejus argus* abundance and presence–absence data as well as the *Lasius niger* frequency data, but a more detailed analysis revealed that it was

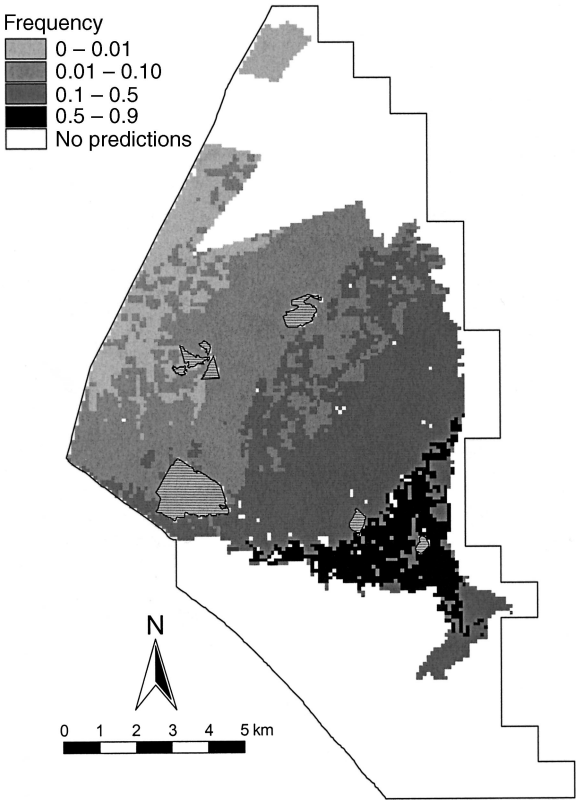

FIG. 5. Potential frequency map of *L. niger* in the *H. halimifolium* scrubland area in the Doñana National Park modeled by the GLM model in Table 4. Values are the predicted proportions of *H. halimifolium* plants with ant nests at the base. The horizontally striped areas are dense *Pinus pinea* plantations, which were excluded from calculations.

not biologically significant. Overall, the correlograms were approximately inverse J-shaped, indicating that the data conform to a bell-shaped surface (Legendre and Fortin 1989). However, after accounting for environmental variables, the significance of the correlograms was lost, apart from a positive correlation of *L. niger* frequencies in sites separated by <3 km (Fig. 4). This suggests that most of the spatial pattern observed in the raw data was due to spatial autocorrelation of environmental variables. We actually do not know the underlying mechanisms of the autocorrelation in residual *L. niger* frequency. Neighborhood effects appear to be an unlikely possibility, because the calibration 100-m grid squares was subject to the condition of being located in different 1-km UTM squares and were ≥600 m apart. Because of their social organization, ant colonies are considered as relatively static entities that can only survive in habitats that are permanently suitable for them (Boomsma and de Vries 1980). Thus, it seems unlikely that the probability of *L. niger* frequency in a given grid square would be affected by its frequency in neighboring grid squares separated by hundreds of meters of terrain.

Ecological basis of the habitat models

Both observed *P. argus* abundance and presence–absence in 2000 were mostly related to *L. niger* frequency, and to a lesser degree to other environmental variables (curvature, easting, presence of heathland, slope), as shown by the GLM models in Tables 2 and 3, but not with predicted *Halimium halimifolium* density. This is in line with other studies in Doñana National Park and elsewhere, in which *P. argus* abundance and presence were positively correlated with *Lasius* ant abundance rather than host plant cover (Ravenscroft 1990, Jordano et al. 1992). *P. argus* larvae are clearly recognized by *Lasius* ants (and in contrast are attacked by other ant species), and take refuge and pupate in the ant nests; in turn, *Lasius* ants feed on the secretions produced by the larvae, apparently establishing a net mutualistic relationship (Rodríguez et al. 1991, Jordano and Thomas 1992). *P. argus* eggs are disproportionately laid on and around the stems of *H. halimifolium* plants associated with *Lasius* ant nests, and this spatial relationship of the butterfly with ant nests becomes closer for larvae and pupae (Jordano et al. 1992). In addition, *L. niger* appears to be an important cue in determining the movement patterns of adult *P. argus* (Seymour et al. 2003). Although there is no empirical study that explicitly shows whether there is differential survival between eggs, larvae, and pupae on host plants with and without *Lasius*, all data suggest that *Lasius* ant frequency along with the presence of suitable host plants is probably the most important determinant of habitat quality for *P. argus*. But what determines habitat quality for *Lasius* ants?

The mutualistic ants of *P. argus*, *Lasius alienus*, and particularly *L. niger*, are widely distributed species in Europe, even living close to, or in, human habitations.

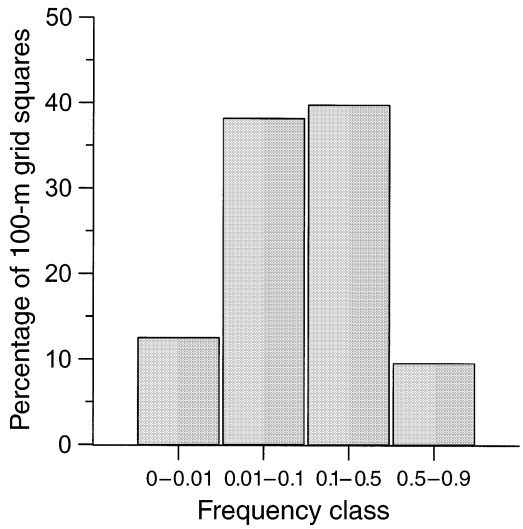

FIG. 6. Relative frequency distribution of 100-m squares in different *L. niger* frequency classes mapped in Fig. 5 and based on the GLM model in Table 4 (N = 9236 squares).

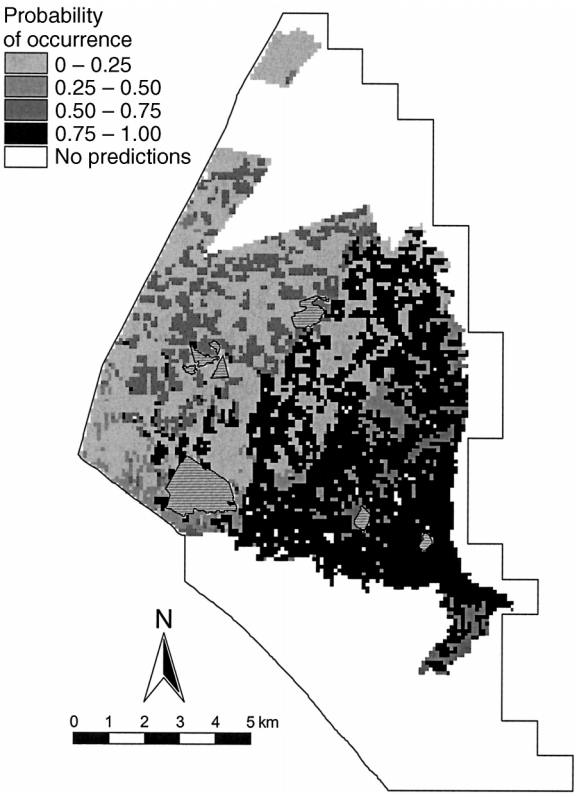

FIG. 7. Probability-of-occurrence map of *P. argus* in the *H. halimifolium* scrubland area in the Doñana National Park predicted by the GLM model in Table 3a. The horizontally striped areas are dense *Pinus pinea* plantations, which were excluded from calculations.

Nevertheless, *L. niger* shows a particular preference for those habitats with relatively high soil humidity; particularly in southern Spain, this species often occurs in riverbanks and marshes (Luque García et al. [2002], and references therein). This fits well with the environmental factors that significantly predict *L. niger* frequency in the GLM model: elevation and presence of heathland border. Both variables probably have no direct effect on *L. niger* frequency, but act as surrogates for the level of soil water content. In the Doñana dune system, water table depth is inversely correlated with elevation, lower sites being wetter than higher sites. In turn, in the mediterranean scrubland area, heathland vegetation occupies those locations in which there is more water availability over the season (Rodríguez et al. 1994). Thus, the GLM habitat model suggests that *L. niger* is associated with the wettest habitats in the scrubland in Doñana, at least in the range of sites investigated. *L. niger* also occupies habitats containing high soil moisture elsewhere in Europe (Brian 1964, Gallé 1975, Boomsma and de Vries 1980, Boomsma et al. 1982).

Evaluation of the habitat models

Most of the GLM models developed in our study accounted for a significant amount of deviance (30–72%) in *P. argus* abundance and presence–absence and in *L. niger* frequency. At first, these results suggest that our models performed well at identifying the habitat quality for the butterfly and its mutualist ant. Nevertheless, we failed at finding a significant model for *P. argus* abundance using variables from remote sensing only (excluding *L. niger* frequency, Table 2c). In addition, when we implemented our models on a set of independent evaluation sites, the models varied in their levels of performance. The model for *P. argus* abundance, despite explaining a large amount of deviance (72%) in cells used to derive the model, gave rather weak predictions of *P. argus* abundance in evaluation cells when using observed *L. niger* frequencies, and very unreliable predictions when using predicted *L. niger* frequencies in the calculations (Table 5). In this case, *P. argus* abundance predicted from a model including *L. niger* frequency only, or simply the predicted frequency of the mutualist species *L. niger*, was better correlated with observed *P. argus* abundance than with values from the more complex model including all significant variables. This fact suggests that some caution should be taken when interpreting independent variables in habitat models, particularly if no proper evaluation tests are subsequently made. It could be possible that some variables that significantly accounted for some deviance in *P. argus* abundance in the formulation data set actually have no biological significance. This problem appeared to arise when we evaluated the model using an independent set of localities.

The models for *P. argus* presence–absence were much more robust than that for abundance. Despite accounting for moderate amounts of deviance in the calibration cells (30–53%), they had a high discriminatory ability between occupied and unoccupied sites

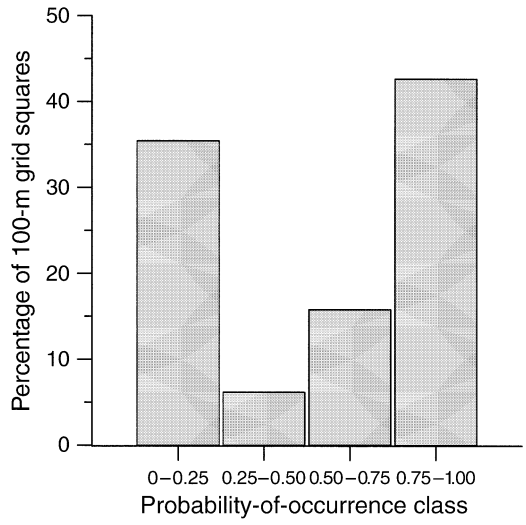

FIG. 8. Relative frequency distribution of 100-m squares in different *P. argus* probability-of-occurrence classes mapped in Fig. 7 and based on the GLM model in Table 3a (N = 9236 squares).

in the evaluation cells, regardless of whether observed or predicted *L. niger* frequencies were used in the calculations. Thus, these models appear to be a sound tool for generating high-resolution habitat maps like that shown in Fig. 7. In this case, even though predicted values from a model with *L. niger* frequency only, or simply *L. niger* frequency, were both significantly related to *P. argus* presence–absence, the whole model, including additional environmental variables (Table 3a), accounted for a greater percentage of the deviance observed in the evaluation test (Table 5). Thus, the presence–absence model may have included independent variables with more biological significance than the abundance model. Surprisingly, the model with all significant variables other than *L. niger* frequency (Table 3c) had a similar predictive ability to the whole model. However, this model was mostly based on geographical position variables (northing and easting), making difficult any interpretation and, consequently, any further applicability. It is worth mentioning here that, although model tests were made using independent data sets, they were located within the same geographical area as the calibration sites. The more general applicability for our model outside the study area would need to be confirmed with more data from other regions of the species distribution (e.g., Mladenoff 1999, Luck 2002*a*, *b*).

To conclude, we have shown here that predictions from a model for the frequency of an ant species can be better correlated with the abundance of its obligate mutualist associate than predictions from a more complex model including ant frequency as well as additional topographical variables. In any case, the significant positive (but moderate) relationships of predicted *L. niger* frequency with *P. argus* abundance and occurrence led to the important insight concerning the possibility of using the predicted distributions of some species to generate those of their associates. In our study system, sampling for butterflies is certainly easier than for ants. However, sampling *P. argus* is extremely time limited because the annual flight period lasts approximately one and a half months, and the flight peak appropriate for sampling only lasts two or three weeks. In addition, transects must be walked during a limited time period within the day. In contrast, *L. niger* ants can be sampled nearly throughout the whole year, except in the coldest (January and December) and hottest months (July and August), and throughout the day, except in the hottest time of day. Using the *L. niger* model, we could delineate the potential *P. argus* habitat distribution within an area before the beginning of the butterfly's flight period. In fact, we used this model to design a release experiment to evaluate the effects of habitat on *P. argus* dispersal (Seymour et al. 2003).

Our approach could be applicable to cases other than mutualist systems where the modeled species is important and easier to survey in the field than its associate. One promising possibility is herbivore insects, for which, in many cases, host plants provide most resources for larvae and adults. Plants are frequently easier to record in the field than insects, because plants can be spotted for a longer period within the season and at any time in the day. Nevertheless, further research is needed on this approach to evaluate the generality of modeling mutualist or host plant species distributions to predict those of their associates.

Applicability of the habitat models

One of the habitat models derived in our study allowed us to construct maps delineating the spatial distribution of *P. argus* in terms of probability of occurrence and, consequently, to obtain an estimate of the amount of habitat within different quality categories. The model showed that a large percentage (42%) of the landscape containing host plants is unsuitable for the butterfly, suggesting highly heterogeneous habitat quality for this species within an apparently homogenoeus region. The derived map, along with the habitat model, would be useful in assessing the consequences of past or future habitat changes or possible management plans on the distribution of *P. argus*. For instance, major changes in land use around the Doñana National Park since 1970 have generated important alterations in the water table depth. Well construction and the transformation of scrubland into agricultural land have noticeably increased the net water requirement in the area. The huge tourist urbanization in Matalascañas (Fig. 1) has also contributed directly to the decrease of the water table (Fernández-Delgado 1997). For instance, withdrawal of groundwater beyond the Park boundaries produced a water table depth decline rate ranging from 0.1 to 0.3 m/yr during the 12-year period from 1975 to 1987 (Suso and Llamas 1990). Research has already shown some vegetation changes (e.g., a decline in heathland area) as a result of a decrease in water availability (Zunzunegui et al. 1998, Muñoz-Reinoso 2001), but its impact on insect fauna, particularly on *L. niger* and *P. argus*, still needs to be evaluated. Although *P. argus* is not currently a species of conservation concern in Spain, we could evaluate whether widespread butterfly species in Spain are suffering marked declines, as was recently found elsewhere (e.g., León-Cortés et al. 1999, 2000). If so, our results would support the idea of establishing monitoring schemes based on habitat models that assess changes in the status of all species, not only of the rarest (León-Cortés et al. 1999). *Cyaniris semiargus* is another butterfly whose breeding habitat depends on water table depth in Doñana, and it has been hypothesized that it could suffer habitat loss without any significant change in the distribution and abundance of its host plant *Armeria velutina* (Rodríguez et al. 1994).

Our study shows the feasibility of developing habitat models for a target species based on its associate species. The model derived here suggests that even an apparently homogeneous scrubland can be a complex

mosaic of habitat quality for one butterfly, suggesting that the potential impact of habitat changes on its populations will strongly depend on their spatial location. Implementing habitat models for other species, including the incorporation of novel techniques and statistical methods, is an urgent matter if we intend to understand the impact of habitat loss and fragmentation on biological diversity.

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[CAPÍTULO 4]

"WATER AVAILABILITY DRIVES THE HABITAT QUALITY FOR THE BUTTERFLY PLEBEJUS ARGUS IN A MEDITERRANEAN SAND DUNE LANDSCAPE"

“WATER AVAILABILITY DRIVES THE HABITAT QUALITY FOR THE BUTTERFLY PLEBEJUS ARGUS IN A MEDITERRANEAN SAND DUNE LANDSCAPE”

Resumen

Para las especies terrestres, el hábitat es frecuentemente definido como parches físicos de un cierto tipo de cobertura terrestre en una matriz de no hábitat. Sin embargo, la definición apropiada de la calidad del hábitat requiere información más detallada sobre los recursos y condiciones que son necesarios para el mantenimiento de la especie ("enfoque basado en los recursos naturales"). En los artrópodos, su ciclo de vida comprende varias etapas claramente diferenciadas que pueden depender de diferentes recursos y condiciones para el desarrollo exitoso; a su vez, los varones y las hembras pueden tener diferentes requisitos de hábitat debido a sus diferentes comportamientos. Aquí examinamos la calidad del hábitat de machos, hembras y huevos de la mariposa *Plebejus argus* en un paisaje continuo de dunas de arena en el sur de España. Para ambos sexos, la abundancia se relacionó con la densidad de la planta huésped (negativamente) y la distancia a la brezal (un sustituto de la disponibilidad de agua, negativamente para los machos y en forma de joroba para las hembras), y para los hombres sólo, positivamente con la presencia de flores. La abundancia de huevos se relacionó positivamente con la frecuencia mutua de las hormigas y la presencia de flores, y negativamente a la distancia a la brezal. Por lo tanto, los adultos de sexo femenino y masculino y los huevos de *P. argus* en parte diferían en su dependencia de recursos y condiciones, aunque la distancia a la brezal era la variable común para todos los modelos. Esto sugiere que la humedad del suelo es la variable clave para la calidad del hábitat de *P. argus*, probablemente por los efectos directos sobre los individuos y los efectos indirectos en las especies que interactúan (hormigas mutualistas y fuentes de néctar). Como resultado, encontramos una alta congruencia en la calidad del hábitat entre machos y hembras, y una congruencia moderada entre los adultos y los huevos.

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ORIGINAL PAPER

Water availability drives habitat quality for the butterfly *Plebejus argus* in a Mediterranean sand dune landscape

Pilar Fernández[1] · **David Gutiérrez**[2] · **Diego Jordano**[1] · **Juan Fernández Haeger**[1]

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Abstract For terrestrial species, habitat is frequently defined as physical patches of a certain land cover type in a matrix of non-habitat. However, the appropriate definition of habitat quality requires more detailed information on the resources and conditions that are necessary for the maintenance of the species ('resource-based habitat approach'). In arthropods, their life cycle comprises several clearly differentiated stages that may depend on different resources and conditions for successful development; in turn, males and females may have different habitat requirements due to their different behaviours. Here we examine the habitat quality for males, females and eggs of the butterfly *Plebejus argus* in a continuous sand dune landscape in southern Spain. For both sexes, abundance was related to host plant density (negatively) and distance to heathland vegetation (a surrogate for higher water availability; negatively for males and hump-shaped for females); and, for males only, positively with flower presence. Egg abundance was positively related to mutualistic ant frequency and flower presence, and negatively to distance to heathland. Hence, female and male adults and eggs of *P. argus* partly differed in their dependence on resources and conditions, although distance to wetter areas of heathland was the common variable for all models. This suggests that soil humidity is the key variable for *P. argus* habitat quality, probably through both direct effects on individuals and indirect effects on interacting species (mutualistic ants and nectar sources). As a result, we found a high spatial congruence in habitat quality between males and females, and a moderate congruence between adults and eggs.

Keywords *Lasius niger* · Lycaenidae · Nectar sources · Resource-based habitat · Water table depth

✉ Pilar Fernández
bv2ferop@uco.es

[1] Departamento de Botánica, Ecología y Fisiología Vegetal, Universidad de Córdoba, Campus de Rabanales. Edificio C-4, 14071 Córdoba, Spain

[2] Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, 28933 Móstoles, Madrid, Spain

Introduction

The occurrence of species is affected by the area, isolation and quality of habitat patches (Thomas et al. 2001). In continuous landscapes, in which the spatial arrangement of habitat (area and isolation) has little effect on the distribution of a given species, the relative role of habitat quality is expected to be more important (Mortelliti et al. 2010). Vegetation or land cover types are frequently used as surrogates of habitat availability, but the appropriate definition of habitat quality requires more detailed information on the resources and conditions that are necessary for the maintenance of the species (Dennis et al. 2003; Mortelliti et al. 2010). This idea underlies the 'resource-based habitat approach' (Dennis et al. 2003), which has been suggested a much better perspective for efficient conservation of species (Dennis et al. 2006).

Key processes involved in survival and reproduction of an individual should be the most important clues in habitat use (Sharp et al. 1974; Stefanescu and Traveset 2009). These clues include different abiotic factors like climate, geology, nutrients, shelter but also biotic interactions (Kubo et al. 2009; Berg et al. 2013; Lawson et al. 2014; Ochoa-Hueso et al. 2014; Suggitt et al. 2015). In the case of arthropods, their life cycle comprises several clearly differentiated stages that may depend on different resources and conditions for

successful development. Most Lepidoptera require certain food plants during their larval stage and nectar-producing plants, mating and roosting structures during their adult stage (Dennis et al. 2003). In turn, within the adult stage, males and females may have different habitat requirements due to their different behaviour. In the case of Lepidoptera, males will invest substantial time in finding appropriate sites for maximising their mating success, whereas females will invest time in finding the most appropriate places for egg laying (Scott 1975). Hence, the habitat quality of the different stages and male and female adults could be associated to different sets of environmental variables (Öckinger 2008; Örvössy et al. 2014; Roche et al. 2015), and consequently, the habitat for a given species will be defined by the union of these resources and conditions (Dennis et al. 2006; Vanreusel and Van Dyck 2007).

Spatial and temporal variation of resources has a fundamental role in the microdistribution of butterflies in the field (Wiklund and Åhrberg 1978; Stefanescu and Traveset 2009; Kubo et al. 2009). For instance, the spatial arrangement of larval resources (food plants) and adult resources (sources of nectar) constrain the movement of butterflies (Sharp et al. 1974; Murphy 1983; Brommer and Fred 1999; Zhan et al. 2014). If the spatial patterns of these variables are not congruent, this could have consequences for species' occurrence because population dynamics will depend on successful dispersal between the separate sites containing the resources and conditions for the different stages and sexes (e.g., Fred et al. 2006) and on behavioural choices (Auckland et al. 2004). The recognition of the detailed habitat requirements has been achieved for several threatened Lepidoptera (e.g., Vanreusel and Van Dyck 2007; Turlure et al. 2009; Maes et al. 2014; Örvössy et al. 2014), but but this has rarely been made using the same sampling unit and considering the two separate sexes (Odendaal et al. 1989; Schultz et al. 2012).

In this study, we examine the small-scale habitat use of the butterfly *Plebejus argus* in a continuous landscape in southern Spain. This is an appropriate model species for evaluating the resource-based habitat approach because adults occur in high abundance, sexes can be easily distinguished in the field, and eggs are laid in conspicuous batches at host plant bases. The habitat requirements for the different developmental stages of *P. argus* were previously studied over its European range (Jordano et al. 1992), and suggested that *P. argus* eggs, larvae, pupae and adults were consistently spatially associated with mutualistic ants of genus *Lasius*, whereas other aspects of *P. argus* habitat were more variable. However, these findings were based on data from different sources collected at different spatial scales and they did not consider the potential differences between sexes. Our approach here addresses separately the habitat quality for eggs and male and female adults of *P. argus* using comparable data collected over the same space and during the same time period. We hypothesise that the habitat for adult butterflies will be mostly related to the abundance of nectar resources, while the habitat for butterfly eggs will be associated with the abundance of larval host plants and mutualistic ants. Butterfly females are expected to select those habitats where larval resources are more abundant.

Methods

Study system

The silver-studded blue, *P. argus*, is a widely distributed butterfly in Europe and Asia (Tolman and Lewington 1997; Kudrna 2002). In Spain, it is also a relatively common species to the northern half of the country, but in the south its populations are more restricted to mountainous areas (Fernández et al. 2015). One exception is Doñana in southern Spain, where the *P. argus* populations are the southernmost in Europe but they occur at relatively high densities at sea level (Rodríguez et al. 1991; Fernández et al. 2015). There are a large number of larval host plants reported for *P. argus*, including species in the family Cistaceae, Ericaceae and Fabaceae (Tolman and Lewington 1997). The larvae are strongly myrmecophilous, attended only by species of the genus *Lasius*, specifically *L. niger* and *L. alienus* (Jordano et al. 1992) in a typical obligate mutualism (Rodríguez et al. 1991; Jordano et al. 1992). In the interaction, the butterfly larvae obtain the protection of ants and have developed a sugar reward mechanism, as in many other Lycaenidae species (Pierce et al. 2002).

The study was performed in Doñana National Park, located on the southwestern coast of Spain. The landscape in this area is relatively flat and homogeneous, with subregions dominated by different shrub covers ultimately driven by soil humidity (Fig. 1): mobile sand dunes bordering the Atlantic Ocean to the south, an extensive marsh to the east, and scrubland on fixed prehistoric sand dunes to the west and north. Due to the sandy nature of the substrate, the soil in Doñana has an extremely low field capacity, and soil water rapidly drains into the groundwater or is lost by evapotranspiration following the rainy season. Water table depth is related to elevation and, consequently, soil water availability is markedly limited at the highest sites during summer drought period (Custodio and Palancar 1995). Nevertheless, there are also some wet soil areas at high elevations due to local effects associated with the drainage to the bottom of slopes. Thus, there is marked variability in the composition of the Mediterranean scrubland associated with the humidity gradient (Zunzunegui et al. 1998): at the driest sites, the scrubland is dominated by species such as *Halimium halimifolium, H. commutatum, Cistus libanotis, Stauracanthus genistoides* and *Rosmarinus officinalis* (known as 'monte

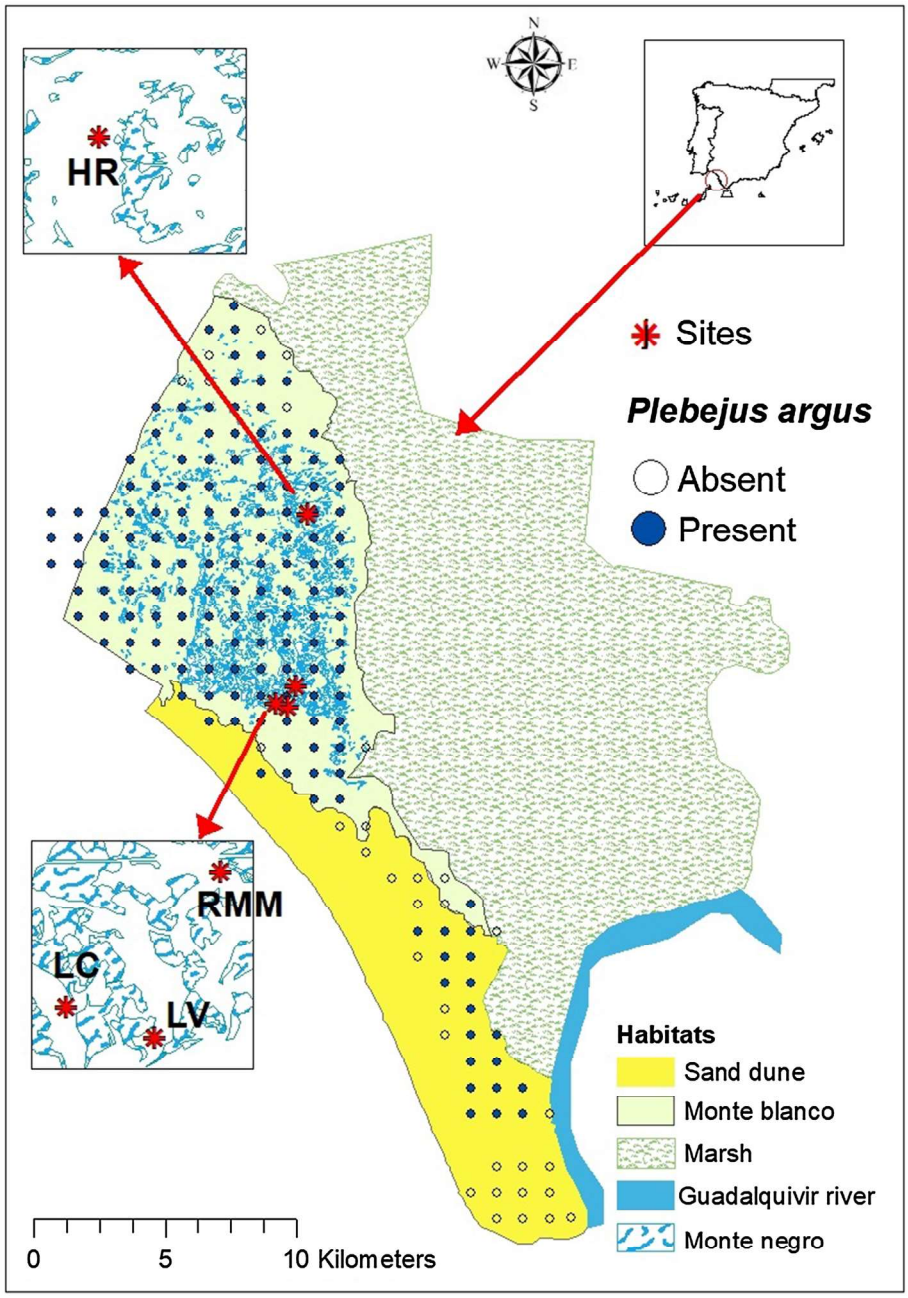

Fig. 1 Map of Doñana National Park, showing the four general types of land cover [marsh, sand dune, heathland ('monte negro') and dry scrubland ('monte blanco')] and the four sampling sites [Hilillo Rosado (HR), Las Verdes (LV), Los Corzos (LC) and Rancho de María Manuela (RMM)]. The presence of *P. argus* at 1 km^2 resolution is also shown (from Gutiérrez et al. 2004)

blanco'), whereas at the wettest sites it is dominated by a heathland community of *Erica scoparia, Rubus* sp. and *Ulex minor* (known as 'monte negro'). Thus, the heathland zones reflect local, inter-mediate and regional groundwater discharges in Doñana National Park (Muñoz-Reinoso and García Novo 2005).

In Doñana, *P. argus* larvae are relatively host specific, feeding on leaves of *H. halimifolium* (Cistaceae) and tended by *L. niger* ants (Rodríguez et al. 1991). Ant nests are built mainly at the base of the food plants (*H. halimifolium*) and other shrub species using their root system as support in the sandy soils of the study area. *P. argus* is the most abundant butterfly species, with a striking explosion of individuals during late spring (late May to early July). The distribution of the butterfly at 1-km resolution shows that the species is present in virtually all areas occupied by scrubland (Gutiérrez et al. 2004). However, there is high variability in abundance between sites at finer resolutions (100 and 10-m), with values ranging more than one order of magnitude between sites located only 50 m apart (Gutiérrez et al. 2004, 2005). At 100-m resolution, *P. argus* abundance was positively associated with *L. niger* frequency, site upward convexity and easting (Gutiérrez et al. 2005).

Sampling

The spatial distribution of *P. argus* was studied during the spring of 1999 at four sites (Fig. 1) (Hililllo Rosado, HR; Las Verdes, LV; Los Corzos, LC; and Rancho de María Manuela, RMM). At each site, we set a permanent transect along the steepest slopes of fixed sand dunes, which showed a vegetation gradient from drier scrubland ('monte blanco') at the highest part towards the border of wetter heathland ('monte negro') at the bottom of slopes, Each transect consisted of several consecutive 10-m^2 plots (10-m plots henceforth) marked with aluminium poles, with the last plot just adjacent to heathland. We were not able to include whole heathland areas in the gradient because their high scrub density prevented establishing and sampling properly 10-m plots. The numbers of squares at each transect ranged between 10 and 16 and they depended on the length and steepness of the corresponding fixed sand dune slope.

Plebejus argus adult abundance was estimated by carefully searching by two observers and recording all individual butterflies categorised by sex within each 10-m plot. The sampling was carried out on sunny days on three different occasions around the approximate peak of the flight season of *P. argus* (from early-to-mid June) and according to standard methodology in butterfly monitoring schemes (Pollard and Yates 1993). Male and female *P. argus* adult abundances were, respectively, the sums of the total number of individuals of each sex recorded on the three sampling occasions.

Plebejus argus egg abundance was estimated from July onwards (after the adult flight season to ensure the absence of further egg-laying events) as the frequency of *H. halimifolium* plants with egg batches (egg frequency henceforth). In Doñana, *P. argus* eggs, the overwintering stage, are placed at the base or on the litter close to the base of *H. halimifolium* plants. We searched for a sample of 30 *H. halimifolium* plants or all plants within the square if there were < 30 plants.

To examine the potential determinants of the distribution of *P. argus* adults and eggs, we collected environmental variables from the field describing the main resources and conditions for the butterfly: host plant, flower and mutualist ant (*L. niger*) abundances, and water availability. Host plant abundance was estimated by recording the number of *H. halimifolium* plants within each 10-m plot (*H. halimifolium* density henceforth). Flower abundance was measured by counting the number of flowering plants of *Echium gaditanum, Armeria velutina* and *Thymus mastichina* (available nectar sources, Herrera 1985) within each square. All those plant species have been recorded as nectar sources for *P. argus* (unpublished data). *Lasius niger* abundance was estimated as the frequency of *H. halimifolium* plants with *L. niger* nests (*L. niger* frequency henceforth). In Doñana, *L. niger* only makes nests around the bases of bushes due to the instability of the sandy soil, so searches were restricted to *H. halimifolium* plants using the same individual bushes as those for estimating egg frequency (Gutiérrez et al. 2005). Water availability was estimated by two surrogate measures, distance to the nearest heathland, and height above the nearest flooded area, both measured from the centre of each plot to the nearest border of heathland and flooded area, respectively.

We were also interested in examining the potential determinants of the distribution of the mutualist species *L. niger*. Because this species has been linked to early stages in succession (Boomsma et al. 1982; Boomsma and Van Loon 1982), we estimated bare ground cover using two 10-m transects set along the maximum slope steepness within each 10-m plot. Bare ground cover was the proportion of the 10-m transect occupied by open sand and litter, and the estimate for each 10-m plot was the mean for the two transects.

Statistical analysis

Plebejus argus adult abundances were square-root transformed for analyses to avoid influential effects of individual sites and to normalise residuals. Because flower abundance had a large proportion of zero values (33 out of the total 46 plots), this variable was transformed to presence–absence, with 'flower absence' as reference value, to avoid statistical bias from a few influential high abundance data.

To quantify the responses in the abundance of *P. argus* adults and eggs, and *L. niger* to environmental variables, we used linear mixed-effects models (LMMs) with the field-measured environmental variables as fixed effects, and 'site' included as random effect. For *P. argus* adult (male and female separately) abundance and egg frequency, the environmental fixed variables were *H. halimifolium* density, flower presence–absence, *L. niger* frequency and distance to heathland. Because the distribution of one life stage may be restricted by the habitat requirements of other life stages, we tested whether the distribution of eggs was dependent on adult resources and vice versa (Öckinger 2008). In addition, our approach using the same variables in the starting models, made them comparable. For *L. niger* frequency, variables were *H. halimifolium* density, distance to heathland and bare ground cover.

We included linear and quadratic terms (subject in the last case to the condition that the corresponding linear term was included in the model) for the condition variables (distance to heathland and bare ground cover) and only linear terms for the remaining resource and biotic interaction variables. We selected only one (distance to heathland) from the two potential variables describing the soil humidity gradient because scatterplots showed stronger relationships of *P. argus* and *L. niger* abundance with that variable than with elevation above the nearest

flooded area, and they were highly correlated (Spearman's rank correlation, $r_s = 0.75$, $P < 0.001$, $n = 46$). No further pair-wise correlations between the remaining independent variables had absolute values higher than 0.7, the most commonly applied threshold for collinearity (Dormann et al. 2013).

We followed the top-down protocol recommended by Zuur et al. (2009) for building mixed models. For each response variable, first we started with models fitted with restricted maximum likelihood (REML) with as many explanatory variables as possible in the fixed component, but with different random structure (i.e., one model without any random effect and another with a random intercept of 'site'). Because these models were nested with respect to their random component, we used a likelihood-ratio test to determine which model was the 'best', controlling for boundary effects (Bolker et al. 2009). Second, to select the model on which inference for each response variable was based, we fitted with maximum likelihood (ML) all possible models that included different combinations of fixed variables and the selected random component found during the first step. The 'best' model was that with smaller Akaike information criterion corrected for small sample size (AIC_c) (Burnham and Anderson 2002). To approximately include the truly most parsimonious model in a candidate set with a 95% chance, we selected (1) those models with ΔAIC_c (AIC_c difference with the 'best' model) values < 6; and (2) within this set, we retained only those models which did not have simpler, higher-ranking variants (i.e. including a smaller number of the same explanatory variables) (Richards et al. 2011). Final 'best' models were refitted using REML and their adequacy was examined by plotting residuals against fitted values (Zuur et al. 2009). Because further research is needed to demonstrate the utility of model averaging (Burnham and Anderson 2002), inference was based on the models included in the candidate set (Richards et al. 2011). Analyses were performed using packages 'nlme' (Pinheiro et al. 2014) and 'MuMIn' (Bartón 2016; R Development Core Team 2015).

Congruence between habitats of males, females and eggs

We quantified the degree of congruence between the habitat qualities of *P. argus* males, females and eggs using correlations. As surrogates of habitat quality, we calculated the modelled values of abundance (males and females) and frequency (eggs) based on the 'best' models obtained from LMMs (see above); then, we calculated the Spearman's rank correlation coefficients between the three potential pair-wise sets of predicted values.

Results

There was a substantial spatial variation of *P. argus* density between and within transects in just a few tens of meters (plots), being abundance higher in the lower elevation plots of each transect. On average, we found 28.4 ± 38.9 (mean ± SD) butterflies (males and females pooled) per 10-m plot (range 3–248 at HR; 5–56 at LV; 11–49 at LC; and 7–36 at RMM). The proportion of plants with ant nests was also highly variable between plots within the same transect, averaging 0.3 ± 0.2 per 10-m plot. We found plots without ant nests along with others with high densities of them (range 0–0.68 at HR; 0–0.80 at LV; 0–0.75 at LC; and 0–1 at RMM).

Distribution of *P. argus* adults and eggs and *L. niger*

For male *P. argus* abundance, the likelihood-ratio test between the model without any random effect and the model with a random intercept was highly significant (likelihood-ratio = 28.98, df = 1, $P < 0.001$), indicating that adding the random effect of site to the model was an improvement relative to a model without the random term. The mixed-effects model with the lowest AIC_c included negative relationships with *H. halimifolium* density and distance to heathland, and a positive relationship with flower presence (Fig. 2; Table 1), suggesting that male *P. argus* abundance was higher in sites with flowers, low host plant density and relatively close to wet areas. The other two, simpler, models included in the candidate set (albeit with $\Delta AIC > 3.5$) supported the effects of *H. halimifolium* density and distance to heathland (Table 1).

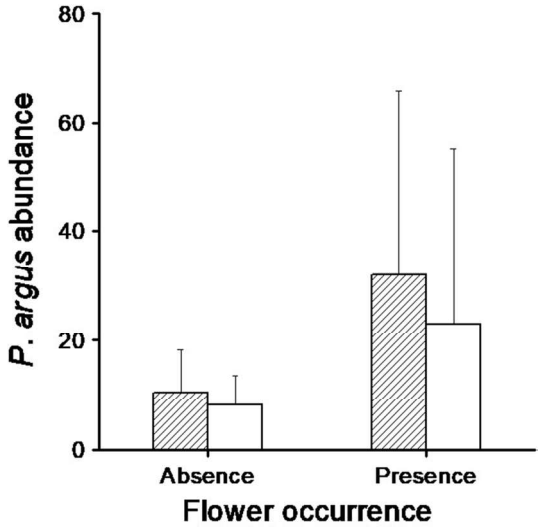

Fig. 2 Mean number (±SD) of *P. argus* adults (males, hatched bars; females, empty bars) in 10-m plots with presence (n = 13) and absence (n = 33) of flowers

Table 1 Candidate set of GLMMs for (a) male and (b) female *P. argus* adult abundance (square-root transformed; n = 46 squares in both cases)

(a) Male *P. argus*

Rank	*H. halimifolium* density	Flower presence	*L. niger* frequency	Distance to heathland	(Distance to heathland)^2	Intercept	K	AIC_c	ΔAIC_c
1	−	+		−		+	6	151.4	0.00
2	−			−	−	+	6	155.2	3.78
3	−			−		+	5	155.3	3.90

(b) Female *P. argus*

Rank	*H. halimifolium* density	Flower presence	*L. niger* frequency	Distance to heathland	(Distance to heathland)^2	Intercept	K	AIC_c	ΔAIC_c
1	−			+	−	+	6	166.0	0.00
2	−			−		+	5	168.5	2.56
3				+	−	+	5	170.5	4.50

The table indicates the fixed variables included in the model and the direction of their coefficients (+/−); number of parameters (K, including two extra parameters for variance and random factor); Akaike information criterion for small sample size (AIC_c); difference in AIC_c between current and best model (ΔAIC_c). Only models within 6 ΔAIC_c units of the top-ranked (lowest AIC_c) model are included. All models include a random factor for 'site' (see "Results" for further details). Flower presence is a categorical variable with 'absence' as reference level

Parameter estimates (±SE) for the best models are: Males: (*P. argus* abundance)$^{1/2}$ = 7.01 (±1.26) − 0.06 (±0.01) *H. halimifolium* density + 1.05 (±0.42) flower presence − 0.04 (±0.01) distance to heathland (m). Females: (*P. argus* abundance)$^{1/2}$ = 5.17 (±1.31) − 0.04 (±0.01) *H. halimifolium* density + 0.01 (±0.02) distance to heathland (m) − 0.0004 (±0.0002) (distance to heathland)^2

For female abundance, there was also a significant random effect of site (likelihood-ratio = 15.38, df = 1, P < 0.001), and the mixed-effects model with the lowest AIC_c included a negative relationship with *H. halimifolium* density and a quadratic relationship with distance to heathland (Table 1). The other two, simpler, models in the candidate set (with $\Delta AIC_c > 2.5$) supported alternatively the negative effects of *H. halimifolium* density and the quadratic relationship to distance to heathland (Table 1).

For *P. argus* egg frequency, model fit significantly improved including a random effect for site (likelihood-ratio = 11.65, df = 1, P < 0.001), and therefore, we used mixed-effects models for this variable. Egg frequency increased with *L. niger* frequency (Fig. 3) and flower presence, and decreased with distance to heathland (Table 2). Nevertheless, there were other closely supported models (ΔAIC_c = 0.24–1.73) including alternatively the effects of flower presence, distance to heathland or neither of those two variables (but all models included *L. niger* frequency) (Table 2).

For *L. niger* frequency, the likelihood-ratio test between the model without any random effect of site and the model with a random effect was not significant (likelihood-ratio = 0.36, df = 1, P = 0.275), indicating that LM instead of LMM was the appropriate approach. The model with the lowest AIC_c included negative relationships with *H. halimifolium* density and distance to heathland (Table 3). The other, and closely supported, model in the candidate set (ΔAIC_c = 0.72) only included the negative effect of distance to heathland, suggesting that closeness to wet areas was the most important variable explaining *L. niger* frequency (Fig. 4; Table 3).

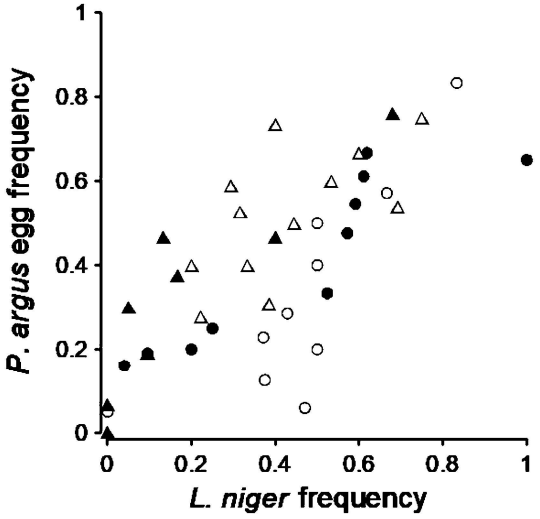

Fig. 3 Relationship between *P. argus* egg frequency and *L. niger* frequency (n = 46 plots). Different symbols represent different sites: Hilillo Rosado, empty triangles; Las Verdes, empty circles; Los Corzos, filled triangles; Rancho de María Manuela, filled circles

Congruence between habitats of males, females and eggs

Plebejus argus males and females showed a very high positive congruence between their habitat qualities (r_s = 0.92,

Table 2 Candidate set of GLMMs for *P. argus* egg frequency (n = 46 squares)

Rank	*H. halimifolium* density	Flower presence	*L. niger* frequency	Distance to heathland	(Distance to heathland)^2	Intercept	K	AIC_c	ΔAIC_c
1		+	+	−		+	6	− 45.5	0.00
2			+	−		+	5	− 45.2	0.24
3		+	+			+	5	− 44.9	0.59
4			+			+	4	− 43.7	1.73

The table indicates the fixed variables included in the model and the direction of their coefficients (+/−); number of parameters (K, including two extra parameters for variance and random factor); Akaike information criterion for small sample size (AIC_c); difference in AIC_c between current and best model (ΔAIC_c). Only models within 6 ΔAIC_c units of the top-ranked (lowest AIC_c) model are included. All models include a random factor for 'site' (see "Results" for further details)

Parameter estimates (± SE) for the best model are: Egg frequency = 0.16 (± 0.09) + 0.08 (± 0.05) flower presence + 0.74 (± 0.08) *L. niger* frequency − 0.002 (± 0.001) distance to heathland (m)

Table 3 Candidate set of GLMs for *L. niger* frequency (n = 46 squares)

Rank	*H. halimifolium* density	Distance to heathland	(Distance to heathland)^2	Bare ground cover	(Bare ground cover)^2	Intercept	K	AIC_c	ΔAIC_c
1	−	−				+	4	−2.2	0.00
2		−				+	3	−1.5	0.72

The table indicates the variables included in the model and the direction of their coefficients (+/−); number of parameters (K, including two extra parameters for variance and random factor); Akaike information criterion for small sample size (AIC_c); difference in AIC_c between current and best model (ΔAIC_c). Only models within 6 ΔAIC_c units of the top-ranked (lowest AIC_c) model are included

Parameter estimates (± SE) for the best model are: *L. niger* frequency = 0.63 (± 0.07) − 0.003 (± 0.002) host plant density − 0.003 (± 0.001) distance to heathland (m)

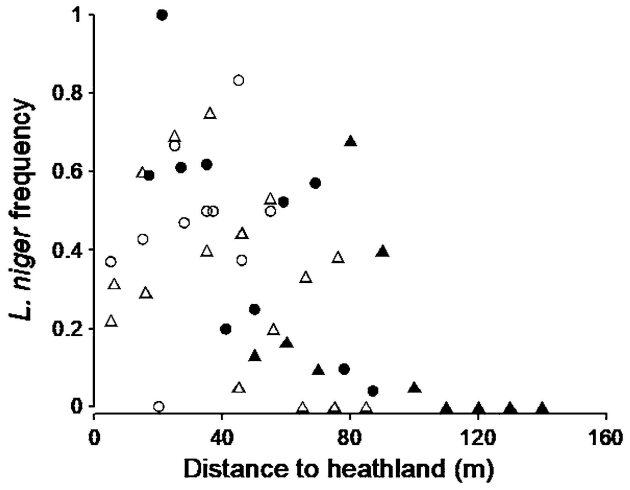

Fig. 4 Relationship between *L. niger* frequency and distance to heathland (m) (n = 46 plots). Different symbols represent different sites: Hilillo Rosado, empty triangles; Las Verdes, empty circles; Los Corzos, filled triangles; Rancho de María Manuela, filled circles

P < 0.001, n = 46; based on modelled abundances, Fig. 5). The relationship between the habitat quality of adults and eggs was also positive and very similar for males and females (r_s = 0.52–0.54, P < 0.001, n = 46 in both cases), but the variability in the modelled egg frequency values increased along the axis of adult abundance (Fig. 5).

Discussion

The adults of both sexes and eggs of *P. argus* partly differed in their dependence on resources and conditions. While the abundance of adult butterflies was negatively associated with host plant density and distance to heathland (and, in the case of males, also positively with flower presence), the abundance of eggs was positively correlated with mutualistic ant frequency (and, in lesser degree, positively with flower presence and negatively with distance to heathland).

Our results show that, although the distances between the most extreme points within each transect could be easily flown by adult *P. argus* (Gutiérrez et al. 2004; Fernández et al. 2016), there was a clearly clumped distribution of adult abundance across plots, ranging up to two orders of magnitude (e.g., HR site). Two variables, distance to heathland and host plant density, were those that best accounted for the variation in abundance of both male and female *P. argus* locally. The abundance of nectar plants (measured as flower presence–absence) was also important (Fig. 2), but it was only included in models for males. *Plebejus argus* is a

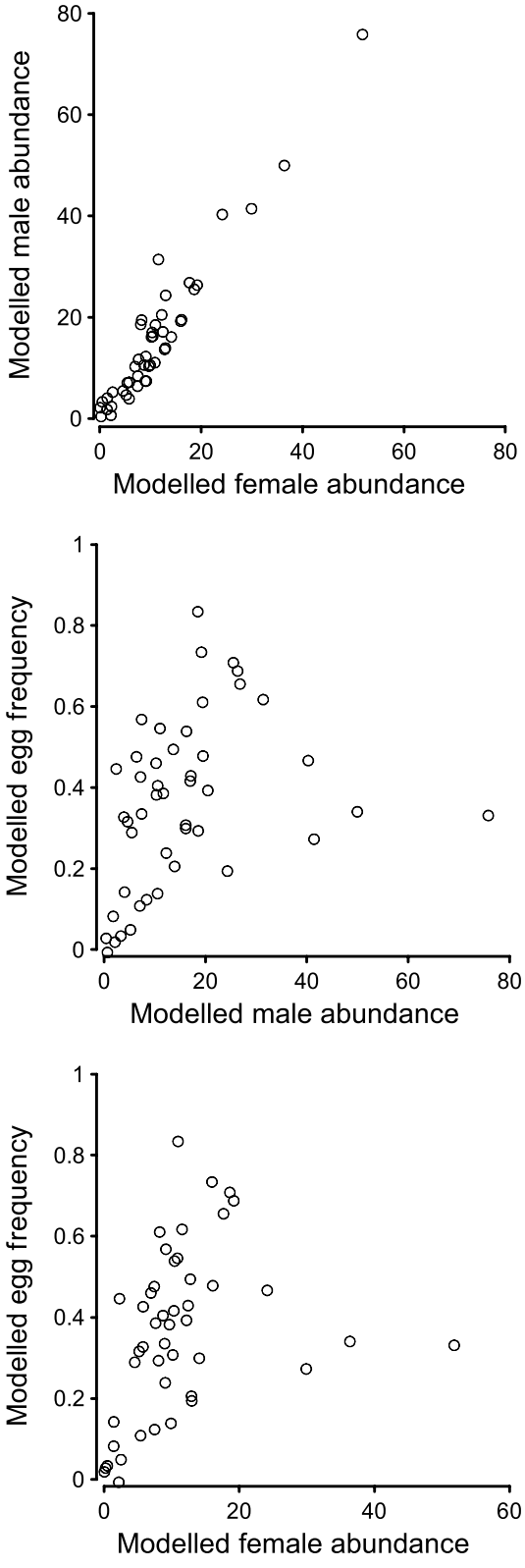

Fig. 5 Pair-wise relationships between modelled male abundance, female abundance and egg frequency. Spearman's rank correlation coefficients (r_s) between modelled variables (n = 46 plots for all cases): modelled male and female abundance, $r_s = 0.92$, $P < 0.001$; modelled egg frequency and male abundance, $r_s = 0.54$, $P < 0.001$; modelled egg frequency and female abundance, $r_s = 0.55$, $P < 0.001$

relatively late-flying species in the context of the sand dune system in Doñana (Rodríguez et al. 1991). This area is characterised by a hot and dry summer, which could mean a high mortality risk from desiccation when the species is on the wing (Mazer and Appel 2001). Therefore, the use of nectar sources and relatively cool and moist microclimates (close to heathlands), probably have a key role in adult survival. This issue could be exacerbated by the fact that, (1) at the peak of the flight period of *P. argus*, the majority of nectar plants are finishing their blooming period in the driest habitats, but not in the transition zones close to heathland (Herrera 1985); (2) during the late flight period, nectar sources are restricted to heathland, represented by *Frankenia hirta, Mentha pulegium* and *Rubus ulmifolius* (Herrera 1985). In addition, high temperature and low humidity in the afternoon force adult *P. argus* to move to cooler areas in heathland, where they aggregate for roosting (personal observation). Unfortunately, as mentioned in the Methods, our sampling design did not include heathland areas to quantify their use. Dennis (2004), although in a very different environment, suggested the importance of shrubs and bushes outside host plant areas for *P. argus* roosting. In this sense, it would be interesting to collect additional behavioural data for evaluating the possible role of heathland as refuge and roosting places for *P. argus* in Doñana.

The surprising negative effect of *H. halimifolium* density on adult abundance suggests that host plants were not a limiting resource at the study sites. This could be expected from the fact that there were no plots without host plants in our study sites, and in most of them, host plants occurred at high density (Örvössy et al. 2014). It is likely the negative effect on *P. argus* abundances to be the consequence of avoidance from areas covered by dense scrubland, as shown in a study in the same area on fine scale movements (Fernández et al. 2016). It could be also hypothesised that the pattern could be the result of a greater host plant density in the driest areas of the transects, but this was not supported because the correlation between *H. halimifolium* density and distance to heathland was low and non-significant ($r_s = 0.24$, $P = 0.110$, n = 46).

Egg abundance showed a somewhat different pattern from adult butterflies, as it was mostly associated with areas with high *L. niger* frequency. This is in line with the previous findings concerning *P. argus* egg distribution patterns (Jordano et al. 1992), and it is the result of the apparent mutualistic relationship between butterflies and ants (Rodríguez et al. 1991). However, it was surprising that *L. niger* frequency did not contribute to the models of adult abundance, as previously shown in a study at coarser resolution and

larger spatial scale (Gutiérrez et al. 2005). Probably, this apparent mismatch was due to the difference between studies in the rates of routine movements to spatial resolutions.

Distance to heathland was the common variable that accounted for butterfly *P. argus* abundance, egg and *L. niger* frequency. In the Doñana sand dune system, water table depth is inversely correlated with elevation, lower sites being wetter than higher sites. In turn, in the Mediterranean scrubland area, heathland patches occupy only those locations in which water availability is higher over the season (Sousa et al. 2012). This suggests that water availability is the key variable for *P. argus*, probably through both direct effects on butterflies (decreasing the risk of mortality by desiccation, Mazer and Appel 2001) and indirect effects on interacting species (mutualistic ants and nectar sources). Accordingly, we found a high congruence in habitat quality between males and females, but a moderate congruence between adults and eggs.

It has been suggested that butterfly populations (and, specifically, *P. argus*) do not occupy the entire space of available resources all the time, because the area of the habitat used can be modulated by weather conditions (Dennis and Sparks 2006). Thus, our results could provide a biased measure of habitat quality just because surveys were undertaken in conditions prescribed for transect recording (Pollard and Yates 1993). Nevertheless, weather conditions are relatively stable (sunny and warm) in Doñana during the flight period of *P. argus*, and hence, the spatial distribution observed in our study probably corresponded to the habitat use during the activity time for most days.

Our results provide further support to our previous concern about the potential vulnerability of *P. argus* and other insect fauna to land use and urbanization in Doñana via water table level alterations (Gutiérrez et al. 2005; see also; Rodríguez et al. 1994; Sousa et al. 2012). Research on temporal hydrological changes suggests that groundwater supply to temporary ponds in Doñana is being reduced by the pumping of subterranean water to a tourist resort close to the national park. This impact has resulted in a decrease in the hydroperiod (time during which a pond holds surface water) for those ponds closer to the resort (Serrano et al. 2008). Associated with the water table level changes, there has been a significant decrease in the area occupied by the more hygrophytic plant communities such as heathlands and pond grasslands (Muñoz-Reinoso 2001; Sousa et al. 2012), which here have been shown to be key indicators for high quality habitat for *P. argus*. Nevertheless, the impact of water table level changes on *P. argus* still needs to be evaluated.

This study shows the importance of habitat quality to understand species' distributions at fine resolutions of tens of meters, with potentially different habitat requirements for the different life stages and sexes. Our results suggest the need to examine the distribution and habitat from a resource-based habitat perspective (Dennis et al. 2003). Only a fraction of the landscape containing host plants was suitable for the species, being necessary the coincidence with other basic resources such as nectar and ants, in turn modulated by water availability. Nevertheless, we found that habitat quality was highly concordant between adult males and females, and moderately concordant between adults and eggs, suggesting that individuals do not need to move long distances between separate resources to complete their life cycle. In any case, this issue should be considered for species management because it may have population dynamic consequences if resources for the different sexes and stages become too isolated between them.

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[CAPÍTULO 5: ESCALA PLANTA]

"HOST PLANT SELECTION IN PLEBEJUS ARGUS (LINNAEUS, 1758) AND ITS MUTUALISTIC ANT. THE ROLE OF PLANT ARCHITECTURE (LEPIDOPTERA: LYCAENIDAE)"

“HOST PLANT SELECTION IN PLEBEJUS ARGUS (LINNAEUS, 1758) AND ITS MUTUALISTIC ANT. THE ROLE OF PLANT ARCHITECTURE (LEPIDOPTERA: LYCAENIDAE)”

Resumen

En el Parque Nacional de Doñana las larvas de *Plebejus argus* (Linnaeus, 1758) se alimentan de *Halimium halimifolium* L. Willk. y mantener una relación mutualista con la hormiga *Lasius niger* (Linnaeus, 1758), anidando en la base de estos arbustos. Se ha analizado la arquitectura de esta planta tratando de evaluar si existen caracteres visuales y estructurales seleccionados tanto por las hormigas asociadas como por las mariposas para la puesta de huevos que permiten un rápido reconocimiento de aquellas con mayor probabilidad de ocupación. También analizamos si el comportamiento de la selección de hormigas coincide en áreas de alta y baja densidad de nidos. Los resultados muestran que las hormigas eligen plantas más altas y más aisladas tanto en áreas de alta como baja densidad de nidos. La selección de la mariposa para la puesta de huevos coincide parcialmente con la de la hormiga, aunque el aislamiento de la planta no tiene importancia para las mariposas. Es posible identificar las plantas objetivo que llevan huevos antes de las claras, tratando de mitigar los efectos colaterales que esta medida de manejo tiene sobre estas especies

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Host plant selection in *Plebejus argus* (Linnaeus, 1758) and its mutualistic ant. The role of plant architecture (Lepidoptera: Lycaenidae)

P. Fernández, D. Gutiérrez, J. Fernández-Haeger & D. Jordano

Abstract

In the Doñana National Park the larvae of *Plebejus argus* (Linnaeus, 1758) feed on Halimium halimifolium L. Willk. and maintain a mutualistic relationship with the ant *Lasius niger* (Linnaeus, 1758), nesting on the base of these shrubs. The architecture of this plant has been analysed trying to assess if there are visual and structural characters selected by both the associated ants and butterflies for egg laying that enable a quick recognition of those with a greater probability of occupancy. We also analyse whether the behaviour of selection of ants matches in areas of high and low density of nests. The results show that ants choose higher and more isolated plants both in areas of high and low density of nests. The selection of the butterfly for egg laying partially matches that of the ant, although isolation of the plant has no significance for butterflies. It is possible to identify egg-carrying target plants prior to scrub clearings, trying to mitigate the collateral effects that this management measure has on these species.
KEY WORDS: Lepidoptera, Lycaenidae, plant architecture, habitat selection, *Plebejus argus*, *Lasius niger*, management, mutualism, egg laying, Spain.

Selección de planta hospedadora en *Plebejus argus* (Linnaeus, 1758) y de su hormiga mutualista. El papel de la arquitectura de la planta (Lepidoptera: Lycaenidae)

Resumen

En el Parque Nacional de Doñana las larvas de *Plebejus argus* (Linnaeus, 1758) se alimentan en *Halimium halimifolium* L. Willk. y mantienen una relación mutualista con la hormiga *Lasius niger* (Linnaeus, 1758), que anida en la base de estos matorrales. La arquitectura de esta planta ha sido analizada tratando de evaluar si hay caracteres visuales y estructurales seleccionados tanto por las hormigas asociadas como por las mariposas para la puesta de huevos que permitan un rápido reconocimiento de los que tienen mayor probabilidad de ocupación. También analizamos si el comportamiento de selección de hormigas coincide en zonas de alta y baja densidad de sus hormigueros. Los resultados muestran que las hormigas eligen las plantas más grandes y más aisladas, tanto en las zonas de alta y baja densidad de hormigueros. La selección de la mariposa para la puesta de huevos coincide parcialmente con la de la hormiga, aunque el aislamiento de la planta no tiene ningún significado para las mariposas. Es posible así identificar plantas con cargas de huevos antes de los tratamientos de aclarado de matorral, para tratar de mitigar los efectos colaterales que esta medida de gestión tiene sobre estas dos especies.
PALABRAS CLAVE: Lepidoptera, Lycaenidae, arquitectura de plantas, selección de hábitat, *Plebejus argus*, *Lasius niger*, manejo, mutualismo, puesta de huevos.

Introduction

In the Doñana National Park (SW Spain) *Halimium halimifolium* L. Willk. (known as

"jaguarzo") is the most abundant scrub species. This species supports the mutualistic relationship established between the butterfly *Plebejus argus* (Linnaeus, 1758) and the ant *Lasius niger* (Linnaeus, 1758), since on one hand it is the main foodplant for the butterfly larvae and on the other it is the physical support necessary for the development of ant nests in this landscape of sandy soils (RODRÍGUEZ *et al*., 1991, JORDANO *et al*., 1992). *P. argus* is a univoltine butterfly that is on the wing from late May to early July. In this protected area (DNP) this butterfly is the most abundant species (FERNÁNDEZ-HAEGER *et al*., 1976) and is present in most of the 1 km^2 grids of the 540 km^2 of the DNP. Its life history is closely linked to that of *L. niger* ants in a typical forced mutualism (RODRÍGUEZ *et al*., 1991; JORDANO *et al*., 1992). The butterfly larvae needs the protection of the ants and, like many other Lycaenidae, has a sugar producing mechanism in reward. Similarly *L. niger* has a wide distribution inside the park (GUTIÉRREZ *et al*., 2005).

The role that egg laying plays in the relationship between plants and insects is basic to understand the ecology and evolution of insects (THOMPSON & PELLMYR, 1991; AGOSTA, 2006). Insect females have developed specific behaviour for ovipositing in locations that are favourable for larval development and their expected survival, thereby increasing their realised fecundity (THOMPSON & PELLMYR, 1991; AWMACK & LEATHER, 2002). In the case of butterflies, host plant selection ranges from plant species to an individual plant within a population, or even specific parts of an individual (HOLEC *et al*., 2006; THOMPSON & PELLMYR, 1991).

Butterflies usually have low mobility larvae and therefore the development of their immature stages depends closely on where females lay their eggs (WAGNER & KARINA, 1997). Although there are errors in the selection of suitable substrates for egg laying, females usually discriminate where to lay the eggs (BROWERS & STAMP, 1987; CLARK & DENNETY, 1988). In batch laying species clutch size determines the importance of a correct choice (COURTNEY, 1984). It can be especially important in species with large clutch size, as success will depend on one or a few decisions, whereas in individual egg laying the female will take multiple decisions and the consequences of a mistake are diluted between the hits. However, in the latter case the female has to develop a behaviour that allows recognising repeatedly the best places for egg laying and survival of offspring.

The selection of the most suitable places is a complex process with a series of steps driven by multiple signals. In a first pre-alighting phase females must find and recognise the host plant following visual or olfactory cues. Later, in a post alighting phase females should evaluate the suitability of the plant to support their progeny. In this phase, females use visual, olfactory, gustatory or tactile cues. Among the visual stimuli, color, shape and height of the plant or branches are crucial, but acoustic communication between ants and imagos can't be ruled out (DENNIS, 1983, 1985; ALVAREZ *et al*., 2005; BEYER & SCHULTZ, 2010; PRADEL & FISHER, 2011). Other stimuli have a chemical origin (REUDLER TALSMA *et al*., 2008; SUWARNO *et al*., 2010), respond to nutrition, palatability or plant age and phenology (RABASA *et al*., 2004; DOAK *et al*., 2006; SUWARNO *et al*., 2010; PATRICELLI *et al*., 2011). Sometimes egg laying spatially responds to the location of plants, as in the use of ephemeral herbaceous species, plants near nectar sources or connectivity between occupied patches (COURTNEY & COURTNEY, 1982; LÖRTSCHER *et al*., 1995; JANSEN *et al*., 2012). This process also addresses inter- or intraspecific antagonistic interactions, such as avoiding competition that sometimes leads to cannibalism among caterpillars and avoiding predators or parasitoids (SCHOONHOVEN *et al*., 1990; BARROS & ZUCOLOTO, 2005; ARAÚJO *et al*., 2006; RIIHIMÄKI *et al*., 2006; OBREGÓN *et al*., 2011).

In addition, the architectural form and complexity of the plant appears to be decisive in species with different trophic relationships (RIIHIMÄKI *et al*., 2006; RUDGERS & WHITNEY, 2006), and its influence can be key by facilitating the emergence of different temperature and humidity microhabitats in a plant (GONÇALVES-ALVIM & FERNANDES, 2001; BEYER & SCHULTZ, 2010). On the other hand, individual plants with a complex structure may facilitate the development of large herbivore populations and, therefore, reduce the probability of extinction, and the maintenance of a diverse invertebrate community (ARAÚJO *et al*., 2006; REID & HOCHULI, 2007).

Many species of Lycaenidae use ants as a signal to oviposit, especially in forced associations (PIERCE & ELGAR, 1985, JORDANO *et al*., 1992; WYNHOFF *et al*., 2008; PATRICELLI *et al*., 2011; JANSEN *et al*., 2012) but respond more weakly to them when the relationship is facultative (PIERCE & EASTEL, 1986). In any case, if the butterflies use the ants as a signal, (THOMAS *et al*., 2009) ant colonies have to be detectable by butterflies and be persistent in space and time.

In the case of ants the appropriate selection of the plant or foraging area may be critical to the success and survival of the colony itself (CAMARGO *et al*., 2003). Habitat selection that ants do may depend on the trunk diameter of the plant, the presence of aphids (PIERCE & ELGAR, 1985; BRISTOW, 1991; SAKATA & HASHIMOTO, 2000) or inter- and intra-specific competitive relationships among others factors (BOOMSMA *et al*., 1982; FOURCASSIÉ *et al*., 2012) and in some specific cases on other physical factors like flood regime (BOOMSMA *et al*., 1982; GUTIÉRREZ *et al*., 2005; HOLEC *et al*., 2006) or microclimate (THOMAS *et al*., 2009). Intraspecific competition for nesting sites seems to be relevant in *Lasius* species, since colonies have fixed nesting sites (BOOMSMA *et al*., 1982).

This relationship is based on the presence of the cistaceae, *H. halimifolium* a widespread shrub species in the DNP, which is the main foodplant of the butterfly larvae and the physical support necessary for the development of ant nests in a landscape of a sandy nature (RODRÍGUEZ *et al*., 1991, JORDANO *et al*., 1992).

In this paper we try to analyse the selection of plants that both ants and butterflies practice and specifically if there are visual cues of the plant that allow us to classify it as occupied or not. In the case of ants we first analyse selection of plants according to their appearance in areas with high density of ant nests. We then try to check if the detected pattern is consistent in areas with low densities of ant nests. We hypothesize a large discrimination in the selection of plants by their morphology in sites where the resource abundance is high, i.e. in places with high availability of plants. Conversely, we also hypothesize that discrimination in plant morphology will be lower in sites where the resource (low plant availability) is limited. For butterflies our model focuses only on areas of high density of ant nests since butterfly egg counting in low density areas did not yield sufficient data for reliable analysis due to the concomitant low abundance of butterflies.

Material and methods

The Doñana National Park (DNP) is located on the southwestern Atlantic coast of the Iberian Peninsula. Much of its surface is covered by scrub colonising former coastal sand dunes (GARCÍA-NOVO *et al*., 1977). The moisture content of the soil and resulting vegetation depends primarily on the height of the dunes over the underlying water table.

We have first analysed the relationship between ants, butterflies and plants in areas with previously known high density of ants and butterflies, where the density of butterfly eggs is enough to allow a reliable assay. During July, when the flying period of this univoltine butterfly is over and eggs are recently laid, we measured 202 plants along two 50 m length transects in two high density places 6 km apart (local scale).

Subsequently, we analysed ant preferences throughout all the DNP, differentiating between areas of high and low density of ant nests. For this purpose we randomly chose 50 squares of 1 ha from all over the park (landscape scale). Each square was subdivided into four equal subsquares and in each three lines of 10 m length were set up (thus twelve 10 m lines for each 1 ha square) (see GUTIÉRREZ *et al*., 2005). We measured a maximum of 10 *H. halimifolium* individuals per line (120 plants per frame). In cases of low density of plants in the square at least 60 plants were randomly chosen.

We checked a total of 4365 plants all over the DNP, 202 of them being in the high-density of ants and butterflies transect.

Three measures (in cm) of the aerial parts of the plant were recorded from each individual of *H. halimifolium*: the height, width (D1_halim) and maximum measurement perpendicular to it

(D2_halim). We also measure the diameter of the stem at the base (D_base). The basal diameter is an easy measure to take in young plants, but not in older ones, since their stem may be creeping or branched. In these cases the whole surface in contact with the sand floor was measured, as an approximation to the surface used by ants to build their nest or butterfly larvae to hide during the day or where they finally pupate. An index of shape was calculated by dividing the height and width.

We also scored the presence of both *Plebejus argus* eggs and *Lasius niger* workers, as well as the degree of isolation of the plant based on the percentage of contact between aerial parts of neighbouring plants (isolation categories: Class 0 (0%), Class 1 (< 25%), Class 2 (25-50%), Class 3 (50-75%) and Class 4 (> 75%).

The relationship between plant variables was analysed by Pearson r correlation test eliminating those with $r > 0.7$. At the local scale, nonparametric tests were used on those variables that could not be normalised (SOKAL & ROHLF, 1984), i.e. degree of isolation or shape, and ANOVA in the remaining ones. The basal diameter was square root transformed to fulfil the assumptions of the analysis. In the detailed analysis of oviposition preferences we conducted a comparison of variance of these morphological variables, considering as factors both the presence of eggs and of ants (2-factor ANOVA).

At landscape scale each 1 ha square was first classified as low or high density, according to the median of ant nest abundance. Later, both the category of ant density and the presence itself were considered as factors in the analysis of preference within a multifactorial analysis of variance with plant height, D2_halim and the square root of the basal diameter as dependent variables. Isolation index does not meet this standard of homoscedasticity and was analysed by nonparametric test (SOKAL & ROHLF, 1984). We selected for the analysis only squares having at least one plant with a nest of *L. niger*.

Results

The correlation test ($n = 4163$, $p < 0.05$) shows that the height of the plant is strongly correlated ($r = 0.71$) with D1_halim and this in turn with D2_halim ($r = 0.91$). Therefore we decided to reject D1_halim from the analyses.

Local scale. High density areas.

Lasius niger plant selection.

Along the two 50 m transects 70 of the 212 plants (33%) checked had an ant nest at the base (70 ant nests in 100 m). Plants with *L. niger* were significantly higher, had bigger aerial parts (bigger D2_halim) and basal diameter than plants without ants (Wilks lambda = 0.92, $F_{3,198} = 6.07$, $p < 0.0001$). The ratio of height / D1_halim (plant shape) was significantly greater in the plants without ants, that is to say, the ant selects wider rather than higher plants, that is, comparatively scrubby ones (Mann Whitney U, $Z = -2.27$, $p < 0.05$). The results of analysis of variance are shown in Table 1.

Table 1.– Mean values with standard deviation of different plant measurements (in cm) according to the presence or not of *Lasius niger* (factor). The F value, significance level, the sum of squares of the effect and the sum of squared error of the different ANOVA analysis are also shown.

	No ants (n=132)	With ants (n=70)	SS effect	SS error	$F_{1,200}$	Significance
Heigth	93.45±26.73	107.60±27.05	9152.81	720.37	12,71	P < 0.0001
D2_halim	77.08±43.39	102.87±46.23	30419.99	1970.37	15,44	P < 0.0001
Sqrt D_Base	4.36±2.14	5.30±1.75	40.97	4.05	10,11	P < 0.005
Shape	1.11±0.59	0.97±0.64				

In addition, there were significant differences in the degree of isolation between plants with and without ant nests, the plants with ants being more isolated (mean value of isolation: 2.06 ± 1.08) than unoccupied ones (average value of class of isolation 1.69 ± 1.16). (Mann Whitney U, Z = -2.06, $p < 0.05$).

Plebejus argus plant selection.

Fifty percent of the plants analysed in the transects had *P. argus* eggs. We also found plants with eggs but without ants (n = 40) and plants without eggs but with ants (n = 9). The probability of finding *P. argus* eggs in plants with *Lasius* is 60.3% and in plants without ants is 39.6%, being this difference significant (X^2: 59.11, df = 1, p = 0.0001).

Regardless of the presence of ants, plants used by *P. argus* for oviposition followed the same pattern as those selected by ants, choosing higher plants with bigger diameter 2 and basal diameter (Wilks lambda = 0.85, $F_{3,198} = 11.65$ $p < 0.001$) (Table 2) the differences between plants with and without eggs being even higher than those with or without ants (see Table 1).

Table 2.– Mean values with standard deviation of different plant measurements (in cm) according to the presence of *Plebejus argus* (factor). The F value, significance level, sum of squares of the effect and the sum of squared of the different ANOVA analysis are also shown.

	No eggs (n=101)	eggs (n=101)	SS effect	SS error	$F_{1,200}$	Significance
Heigth	89.66±25.74	107.05±26.78	15265.03	689.81	22.13	P < 0.0001
D2_halim	69.19±40.68	102.87±44.91	57362.46	1835.66	31.25	P < 0.0001
Sqrt D_Base	4.11±2.078	5.26±1.88	65.88	3.93	16.78	P < 0.0001

However, although there were no significant differences in the degree of isolation of plants (Mann Whitney U. Z = -1.27 $p < 0.1982$), plant shape was different (Mann Whitney U. Z = 3.75 $p < 0.0001$) those with eggs having a lower width/height, ratio that is, a grater development of width in relation to height.

The results of the factor analysis show that plant architecture variables have a significant effect on both the presence of eggs (Wilks test 0.91 $F_{1.196} = 6.58$ $p < 0.0001$) and the combined effect with *L. niger* (Wilks test 0.94, $F_{1.196} = 3.86$ $p < 0.05$) (no intercept model overparameterized Type III). *L. niger* factor is not significant (Wilks test 0.98 $F_{1.196} = 1.57$ ns).

Plants without ants and without *P. argus* eggs were shorter and with a lower basal diameter than plants with eggs, both with ants and without them (Fig. 1). It is striking that plants without ants but with eggs have a bigger basal diameter and are taller than plants without ants or eggs (Unequal N HSD post hoc test Fig. 1). Those plants are attractive to butterflies because of their larger size or even for having nests of *L. niger* until recent times and therefore can still maintain some odorous signals.

There are no significant differences between plants with eggs and no ants and plants with eggs and ants (Unequal N HSD post hoc test Fig. 1). These plants are higher and with bigger diameters than plants without eggs. The low number of plants with ants and without eggs (n = 9) is not enough to allow a proper assessment of this category. The high variability present in all measurements does not allow us to obtain conclusive results on this issue.

Landscape scale. Low and high density areas

In the fifty 1 ha squares examined across the DNP we measured a total of 4,163 plants, 616 of them having *L. niger* ants at the base (14.8%). At this scale there are still significant differences in plants according to the presence of ants, which again are taller, with bigger diameter 2 and bigger

basal diameter (Wilks lambda = 0.97 $F_{3.4159} = 39.79$ $p < 0.0001$). That is, plants with ants, were again more scrubby than those without them.

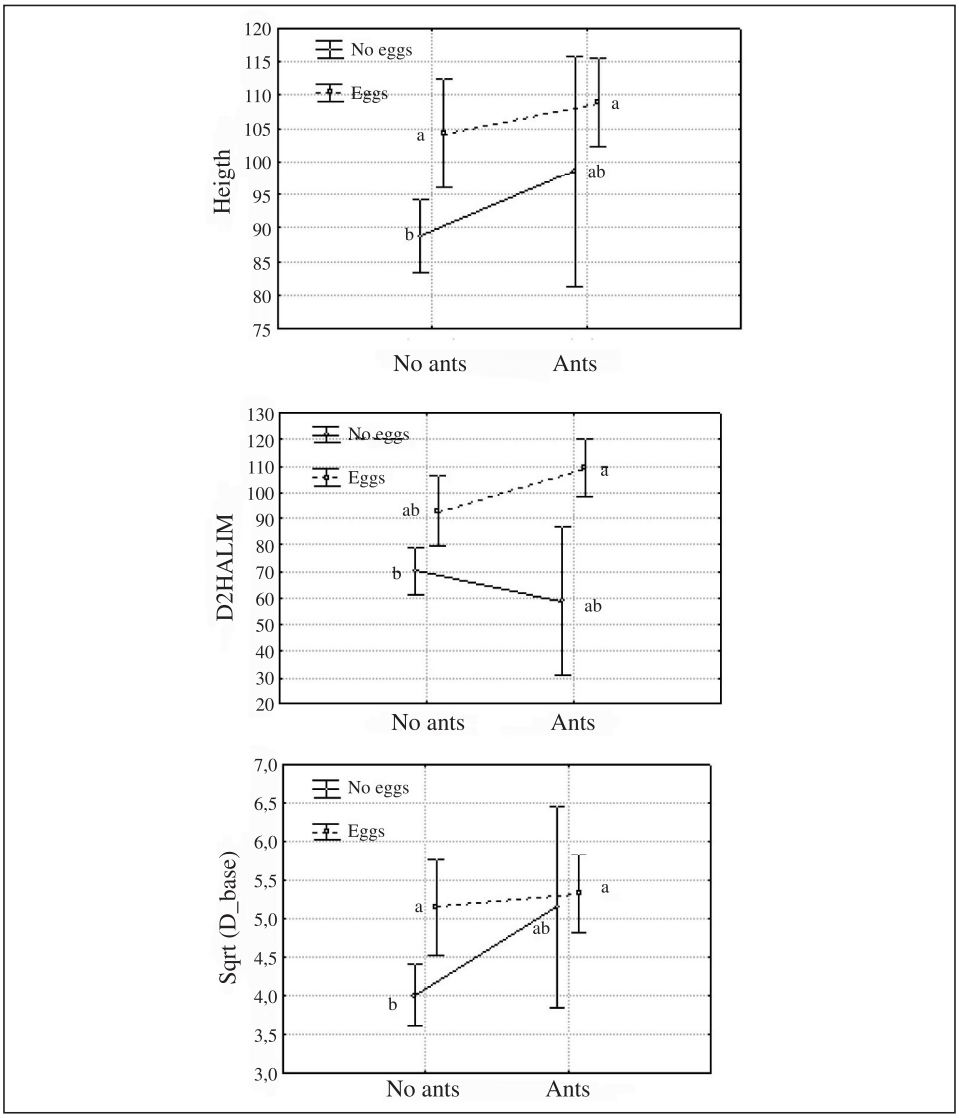

Figure 1.– Mean values and SD of morphological traits of plants according to the presence of ants (*Lasius niger*) and butterfly (*Plebejus argus*) eggs. D2_halim is the maximum length perpendicular to the shrub canopy width. Sqrt (D_base) refers to the square root of basal diameter of the trunk. Letters in graphics represent the results of unequal N HSD post hoc test. Same letters mean no significant differences.

The 50 squares checked for ants have a distribution of frequencies of occupied plants skewed to

the left (Fig. 2), with an average of 0.24 ± 0.22 of occupied plants and a median of 0.1923 (maximum 0.85 and minimum 0.009). Based on the latter value we categorized the squares as high density (0.2 and above), low density (0 to 0.2) or without ants (0). In squares with no ants we counted 0.17 ± 0.07 (mean ± SE) butterflies while in UTM squares categorised as low density ones we counted 7.31 ± 2.29 (mean ± SE) butterflies. This low density of butterfly adults made eggs hard to find on plants and consequently we concentrated the analysis of butterfly preferences only on high density areas.

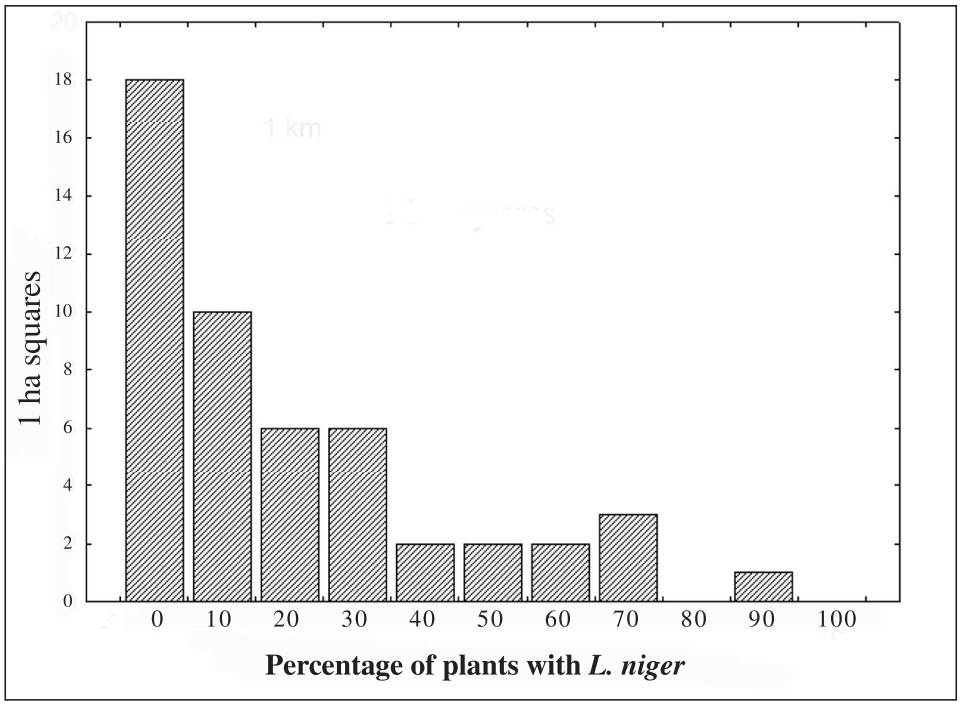

Figure 2.– Numbers of surveyed squares according to the percentage of *H. halimifolium* plants with ants nests at it base.

In squares with no ants (zero density) plants were smaller (mean ± SD, 65.46 ± 35.73) and with a lower basal diameter (mean ± SD, 12.46 ± 16.72) than in the other two density categories. Low density squares had plants with a mean height of 76.09 ± 35.64 (SD) and basal diameter of 14.95 ± 18.64 (SD) while high density squares had plants with a mean height of 71.18 ± 34.38 (SD) and basal diameter of 17.27 ± 19.78 (SD) (Wilks lambda = 0.18 $F_{6.8318}$ = 1894.0 $p < 0.0001$).

In the other two density categories, both the presence of *L. niger* (Wilks lambda = 0.98 $F_{2.2568}$ = 26.17. $p < 0.0001$), the density category (Wilks lambda = 0.98 $F_{2.2568}$ = 26.62 $p < 0.0001$) and the combined effect of both factors (Wilks lambda = 0.99 $F_{2.2568}$ = 3.53 $p < 0.05$) had a significant effect on plant characteristics (significant multifactorial Anova type III decomposition).

The height and diameter 2 in plants with *L. niger* were significantly different in squares with low occupation density (Fig. 3). These plants were taller and with a greater diameter 2 in this low density category, while basal diameter had the greatest value in plants with *Lasius* in high density areas.

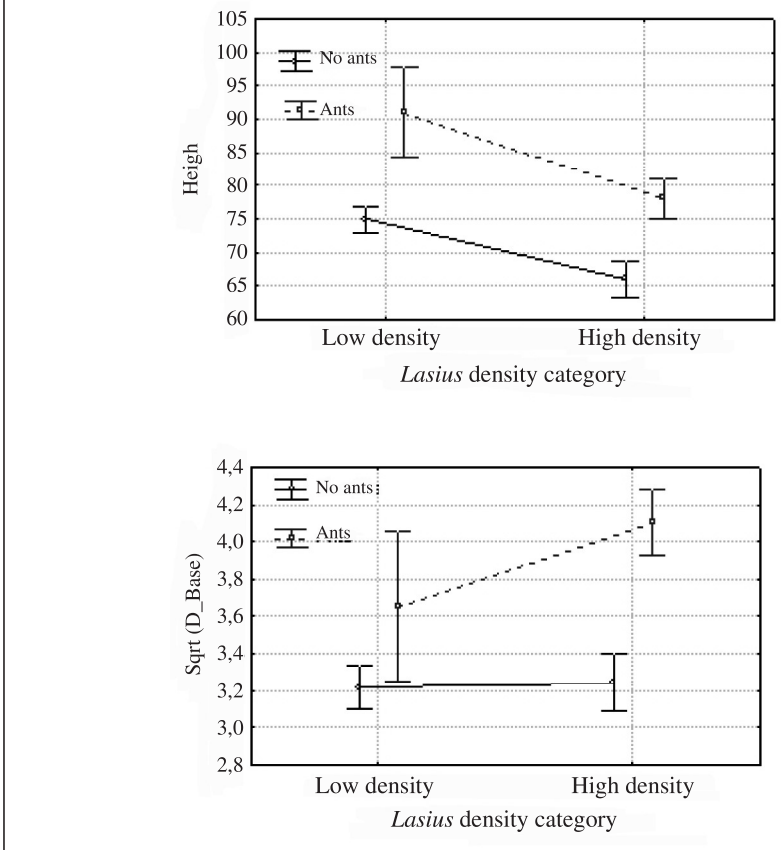

Figure 3.– Mean height and mean basal diameter (square root transformed) (mean ± SD) of the plants with and without *L. niger* in the two ant density categories.

Discussion

The physical support of ant nests is vital in the case of ants that live in extremely dry and adverse locations. The nature of sandy soil in the studied area becomes the limiting factor for building ant nests. *L. niger*, as many other species of ants, uses the roots of the plants as support for building their nests (GUTIÉRREZ *et al*., 2005). The aerial parts of the plant, provide a nest with a more favourable microclimate of humidity and temperature. Furthermore, daily and annual fluctuations of these factors tend to be even more buffered at shallow depth below the sand surface.

L. niger chooses plants with a greater aerial volume and a larger basal diameter. Furthermore, these plants are relatively "scrubby" and less isolated, which suggests a selection of particular plants that ensure a broad and durable physical support to ants in a sandy soil. It is also important to note that these selected large plants must be among the oldest in the area and have been "available" for a longer time for colonisation. Indirectly, the size of these plants indicates that they are in areas that have not

been recently cleared. In addition, the plants selected by ants are in turn less isolated. Ants prefer areas of dense vegetation, where microclimatic conditions allow foraging during summer time when high soil temperature limits ant activity (DOBLAS-MIRANDA & REYES-LÓPEZ, 2008), especially in the pioneering stages of succession to which this ant species is often linked (BOOMSMA & DE VRIES, 1980; BOOMSMA & VAN LOON, 1982). Furthermore, *Lasius* frequently feeds on invertebrates, often found in fragments of vegetation where they, in turn take shelter from high temperatures (HOLEC *et al*., 2006). The degree of isolation may also be related to the use of various plants as support in case of oversized nests that occupy larger areas.

An ovipositing behaviour conditioned by ants has been found in numerous species of butterflies with a myrmecophylic relationship (PIERCE & EDGAR, 1985; PIERCE & EASTEL, 1986; RODRÍGUEZ *et al*., 1991; PIERCE *et al*., 2002; PATRICELLI *et al*., 2011; JANSEN *et al*., 2012). About 75% of Lycaenidae species associate with ants and this association implies specific adaptations of larvae such as the development of ant-associated organs, suppression of ant aggressiveness via specific sounds or mimicking of the pheromones of ant brood (PIERCE *et al*., 2002; ÁLVAREZ *et al*., 2005). Finally, Lycaenidae species whose larvae or pupae live within ant nests for part of their life cycle use ants as oviposition clues (PIERCE & EDGAR, 1985; PIERCE et al., 2002).

In the case of *P. argus*, larvae usually stay during the day sheltered inside ant nests at the base of the plant, protected from high temperatures and predators, beginning to climb the plant for feeding at nightfall (RODRÍGUEZ *et al*., 1991). This behaviour may have evolved in response to the nocturnal foraging habit of *Lasius*, since ants seem to develop an individual behavioural response to temperature and luminosity fluctuations (DEPICKÉRE *et al*. 2008) with different periodic cycles of activity throughout the day that allow foraging in different parts of the habitat at different times of the day or night (BALLARD & PRUESS, 1980). Unlike other species that forage during daytime with high temperatures (CERDÁ & RETANA, 2000) *L. niger* is mainly a nocturnal forager during the summer, when temperatures in the study area at ground level may reach 60 C (unpublished data). In the DNP, *L. niger* presents activity peaks during the hours when temperatures are milder, sundown in summer, noon in winter (unpublished data).

In some butterfly species aggregation of larvae on host plants are considered a visual stimuli for ovipositing females (PIERCE & EDGAR, 1985). However, in this *P. argus* population in southern Spain adults are more active during sunny hours whereas larval aggregations prefer feeding at night. Therefore female host plant selection cannot respond to larvae. Furthermore timing coincidence between adults and larvae along the life cycle is short, suggesting that other signals apart from larvae could make an individual plant attractive to females during their flying period. Gravid females have been observed touching the plants several times before landing on the leaf litter on the bush base someway evaluating the quality of plants for oviposition. As a result plants selected by gravid females coincide to a large extent with the type of plants used by the ants.

P. argus females lay their eggs preferably on plants with *L. niger* and therefore the morphology of the selected plants is adjusted to those selected by ants (greater height and basal diameter, "scrubby" and less isolated plants). However, the difference between the diameters of the plants with and without eggs is bigger than the diameters of plants with and without ants, suggesting that butterflies choose plants with a higher aerial part which, in principle, must guarantee greater availability of resources (leaves, buds and flowers) for larval development. This pattern is consistent both at the local and landscape scale.

Therefore, it appears that the butterfly responds not only to the presence of ants, but also evaluates visually the potential of the plant to feed its progeny. Given the high burden of caterpillars that has been observed (more than 200 on a single plant, unpublished data) a wrong choice could lead to defoliation of the plant and force a shift to neighbouring plants with the risks involved (ANTHES *et al*., 2003; LIU *et al*., 2006). In an observational study of movement, females have been observed performing short flights around a shrub touching the plant several times before landing on the base. The sequence followed should be first a visual evaluation of the plant and then a perception, of the presence of *L. niger* ants.

The foraging area in incipient ant nests is usually small and ants often leave little scent marks in their path. However, mature colonies can cover a large area and actively mark their tracks (MAILLEUX *et al*., 2003). There is also considerable individual variation in the frequency and use of marking (BECKERS *et al*., 1992). Therefore, plants with eggs but no ants could be plants adjacent to other plants with nest within their home range which therefore maintain their fragrant signals. By touching host plants with their antennae butterflies may even be able to recognise the chemical traces left by worker ants (PIERCE & EDGAR, 1985). A successful offspring on these plants will depend on the proximity to a nest of a neighbouring plant and the foraging frequency of ants on it. In these cases, the aerial parts are significantly larger than in others, which further strengthen the idea of selection of plants by different signals other than merely the existence of ants.

Zero density areas had the smallest plants, whereas plants in low density areas are the tallest, suggesting that areas with a recent colonisation by plants are rejected by both ants and butterflies. In those areas of low density of occupation, plants with *Lasius* are significantly higher than plants in the high density zones, that is, ants choose the bigger plants they can find in those low quality areas to start colonisation.

In the DNP clearing of old scrub areas is a valuable management tool that has been used since ancient times. This scrub clearing promotes the appearance of ephemeral grassland and favours shrub regeneration, increases diversity and primary production, and is a basic management measure that positively affects rabbit populations, depleted by viral hemorrhagic pneumonia (MORENO & VILLAFUERTES, 2005). However, actions such as mowing or scrub clearing in the habitat of butterflies have an effect on their populations and may require specific dampening measures (ANTHES *et al*., 2003; STRAUSZ, 2012; BERGSTRÖM, 2005; FARTMANN & TIMMERMANN, 2006; JOHST *et al*., 2006; EICHEL, S. & FARTMANN, 2008). In our study plant architecture provides a visual method for differentiating plants used by *P. argus* and *L. niger*, becoming a simple and easy management tool useful for decision making processes in clearing and cutting of scrub areas. Saving visually small stands of habitat during the process of scrub clearing, keeping plants with greater chance of being occupied by both ants and butterflies and maintaining small nuclei of them could greatly improve conservation of the plant itself and butterfly, accelerating recovery and colonisation.

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P. F., *J. F-H. & .D. J.
Departamento de Botánica, Ecología y Fisiología Vegetal
Universidad de Córdoba
Campus de Rabanales
E-14071Córdoba
ESPAÑA / *SPAIN*
E-mail: bv2ferop@uco.es
E-mail: bv1jobad@uco.es
*E-mail: bv1fehaj@uco.es

D. G.
Área de Biodiversidad y Conservación
Escuela Superior de Ciencias Experimentales y Tecnología
Universidad Rey Juan Carlos
E-28933 Móstoles (Madrid)
ESPAÑA / *SPAIN*
E-mail: david.gutierrez@urjc.es

*Autor para la correspondencia / *Corresponding author*

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BLOQUE II.-EL MOVIMIENTO DE *P. ARGUS*

[CAPITULO 6.]

“FINE SCALE MOVEMENTS OF THE BUTTERFLY PLEBEJUS ARGUS IN A HETEROGENEOUS NATURAL LANDSCAPE AS REVEALED BY GPS TRACKING”

“FINE SCALE MOVEMENTS OF THE BUTTERFLY PLEBEJUS ARGUS IN A HETEROGENEOUS NATURAL LANDSCAPE AS REVEALED BY GPS TRACKING”

Resumen

El estudio del movimiento de las mariposas se ha centrado en el comportamiento de dispersión en el marco de la persistencia de la población en paisajes heterogéneos. La importancia ecológica de los movimientos rutinarios ha recibido menos atención. Estos movimientos pueden estar influenciados por los atributos estructurales de los fragmentos de hábitat, que pueden reflejar la distribución de los alimentos, los conspecíficos, las plantas huésped o las interacciones ecológicas. La influencia relativa de los factores estructurales y funcionales sobre los patrones de vuelo es poco conocida, en parte porque los movimientos de las mariposas se describen a menudo mediante representaciones simplificadas de trayectorias reales. Utilizando el seguimiento GPS de alta resolución obtuvimos trayectorias precisas de movimientos rutinarios de *Plebejus argus* en un paisaje natural heterogéneo. La calidad del hábitat en los parches se clasificó de acuerdo con la abundancia de plantas huésped y néctar, así como la abundancia de nidos de su hormiga mutualista *Lasius niger*. Los movimientos eran lentos y sinuosos en hábitats de alta calidad, mientras que los vuelos más rápidos y rectos se observaron en hábitats pobres. En los bordes, las mariposas cruzan a menudo sin ningún tipo de comportamiento exploratorio hacia parches de mejor calidad, lo que sugiere que pueden usar señales para detectar recursos a cierta distancia. Por el contrario, los individuos normalmente se quedaron en el parche después de explorar los bordes con otros parches de menor calidad. Sin embargo, el escaneado también precedió a las salidas hacia hábitats claramente inadecuados, compatibles con transferencias a parches distantes de alta calidad. Se concluye que los patrones de movimiento en *P. argus* se explican por la heterogeneidad espacial definida por criterios funcionales más que estructurales. También mostramos que los receptores GPS portátiles de bajo costo permiten representar trayectorias de vuelo detalladas en terreno plano abierto revelando patrones de comportamiento complejos

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Fine Scale Movements of the Butterfly *Plebejus argus* in a Heterogeneous Natural Landscape as Revealed by GPS Tracking

Pilar Fernández[1] · Alejandro Rodríguez[2] · Rafael Obregón[1] · Sergio de Haro[1] · Diego Jordano[1] · Juan Fernández-Haeger[1]

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Abstract The study of butterfly movements has focused on dispersal behaviour in the framework of population persistence in heterogeneous landscapes. The ecological significance of routine movements has received less attention. These movements may be influenced by structural attributes of habitat patches or may reflect the distribution of food, mates, host plants or ecological interactions. The relative influence of structural and functional factors on flight patterns is poorly understood, partly because butterfly movements are often described by simplified representations of actual trajectories. Using high-resolution GPS tracking we obtained accurate trajectories of routine movements of *Plebejus argus* in a heterogeneous natural landscape. Habitat quality in patches was ranked according to the abundance of host and nectar plants as well as the abundance of nests of its mutualistic ant *Lasius niger*. Movements were slow and winding in high quality habitats whereas faster, straighter flights were observed in poor habitats. At edges, butterflies often crossed without any exploratory behaviour towards patches of better quality, suggesting they may use cues to detect resources at some distance. Conversely, individuals usually stayed in the patch after exploring edges with other patches of lower quality. However, scanning also preceded exits towards clearly unsuitable habitat, compatible with transfers to distant high-quality patches. We conclude that patterns of movement in *P. argus* were explained by spatial heterogeneity defined by functional rather than structural criteria. We also show that inexpensive

✉ Pilar Fernández
bv2ferop@uco.es

[1] Department of Botany, Ecology and Plant Physiology, University of Córdoba, Campus de Rabanales C-4, 14071 Córdoba, Spain

[2] Department of Conservation Biology, Estación Biológica de Doñana-CSIC, Américo Vespucio s/n, 41092 Sevilla, Spain

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handheld GPS receivers allow depicting detailed flying trajectories in open flat terrain revealing complex behavioural patterns.

Keywords Butterflies · habitat quality · heterogeneous landscapes · high-resolution GPS tracking · routine movements

Introduction

The movement of organisms is a complex process that depends on both internal factors (motion and navigation capacities, motivation to move) and features of the landscape through which they move (Nathan et al. 2008). Landscape variables that influence movement of animals include the spatial distribution of resources, mating opportunities, competitors and natural enemies (Corbett and Rosenheim 1996; Kindvall et al. 1998; Söderström and Hedblom 2007; Woodroffe 2011). Heterogeneity in the distribution of these variables may help to explain patterns in the trajectories followed by individuals moving across mosaic landscapes. In general, higher speeds and straighter trajectories are expected when animals move through patches of low quality, whereas individuals may spend more time along more erratic paths while exploring valuable sites (Dover 1997; Schultz and Crone 2001; Dennis 2004; Baguette and Van Dyck 2007).

Many butterfly species are habitat specialists and, therefore, good models to study the effect of habitat fragmentation on movement (Baguette and Mennechez 2004; Stevens et al. 2010). In most cases, long-distance movements have been studied in the context of dispersal in order to understand population persistence in heterogeneous landscapes under the metapopulation paradigm (Hill et al. 1996; Haddad 1999; Delattre et al. 2010; Weyer and Schmitt 2013). Although the sum of many short-term displacements may also end up in a dispersal event (Van Dyck and Baguette 2005; Hawkes 2009; Franzén and Nilsson 2012), the ecological significance of routine movements has received less attention. These movements may help to explain preference for resources such as food, mates, host plants or physiologically suitable environments (van Dyck and Baguette 2005; Muriel and Kattan 2009; Severns and Breed 2014). Functional components of habitat quality are not always well identified and taken into consideration when defining the boundaries of suitable patches. Crossing decisions of butterflies at patch edges can help understand whether patches described by researchers on the basis of structural attributes are perceived as functional patches, actually differing in quality, by butterflies (Dennis and Sparks 2006; Schultz et al. 2012).

The behavioural response of organisms to heterogeneous environments can be better described when movements are measured with high spatial resolution (Nathan et al. 2008; Getz and Saltz 2008). In butterflies, however, movement patterns are frequently analyzed from quite simplified representations of actual trajectories. The highest level of simplification results from the popular mark-release-recapture experiments (Hill et al. 1996; Brommer and Fred 1999; Gutiérrez et al. 2004; Stevens et al. 2010), where a small number of points are recorded for each individual, sometimes on different days. Following individuals and dropping a few marks along selected points of the flight path is a more appropriate technique to describe routine movements, yet this method reproduces the trajectory imprecisely and may be impractical for long lasting flights (Conradt et al. 2000; Dover and Fry 2001). Another widely used method consists of

estimating the trajectory through linear segments connecting marked points where butterflies land after a flying bout (Korösi et al. 2008; Kalarus et al. 2013; Skórka et al. 2013). These trajectories may be enriched by marking additional points at fixed intervals (Schultz 1998; Kuefler et al. 2010; Kallioniemi et al. 2014). The use of harmonic radar (Riley et al. 1996) extends the range of detection up to 1 km and allows longer trajectories to be recorded with high accuracy (Kissling et al. 2014). Harmonic radar has been successfully used with Nymphalid butterflies (Cant et al. 2005; Ovaskainen et al. 2008). Notwithstanding, this technique also has a number of limitations. First, the microwave signal is attenuated by woody vegetation and terrain, so in practice its use is restricted to open landscapes. Second, long detection ranges are achieved only with the expensive stationary, ground-based version of the radar station (Kissling et al. 2014). Finally, transponders attached to the insect body do not allow individual identification and, despite being tiny (<20 mg, Kissling et al. 2014), only larger species can carry these devices (Van Dyck and Baguette 2005). Radio telemetry has also been used in large insects (Hedin and Ranius 2002; Vinatier et al. 2010) but battery powered radio-transmitters are too heavy for most butterfly species (Kissling et al. 2014). Therefore, there is room for improvement of methods to precisely delineate butterfly flight paths.

Our aim is twofold. First, we study the butterfly *Plebejus argus* as a model to test a novel and inexpensive method for accurately mapping butterfly flight trajectories during routine movements based on high-resolution GPS tracking. We compare patterns of movement emerging from these trajectories with simplified trajectories obtained with one conventional method. Second, we hypothesize that patterns of movement reflect the butterflies' habitat preferences. In habitats where nectar sources, larval host plants, mutualistic species and mates are highly dispersed, butterflies are expected to move faster, with lower stop frequency, and lower sinuosity than in habitats where these resources are abundant. If butterflies are able to detect marked changes in habitat quality we predict that *P. argus* will readily cross into high quality patches but will avoid crossing edges towards patches of lower quality. If an edge crossing takes place, we predict that the pattern of movement will shift according to the change in habitat quality.

Material and Methods

Study Area

The study was carried out in the Doñana National Park, a sandy coastal area of south-western Spain (36°58′46″ N, 6°26′48″W). Observations were made on 1.7-km^2 flat plot (altitude: 7–10 m.a.s.l.) covered with scattered shrubs up to 2 m high growing on stabilized dunes and adjacent mesic depressions (Fig.1). The relatively open structure of this landscape facilitates detection and tracking of butterflies. Dune morphology determines small-scale spatial variation in topography and subsequent variation in the distribution of shrub species. Structure and composition of shrub communities change drastically within tens of meters, depending on the depth of the water table (Muñoz-Reinoso 2001). In zones closer to ground water several heather species occur (*Calluna vulgaris*,

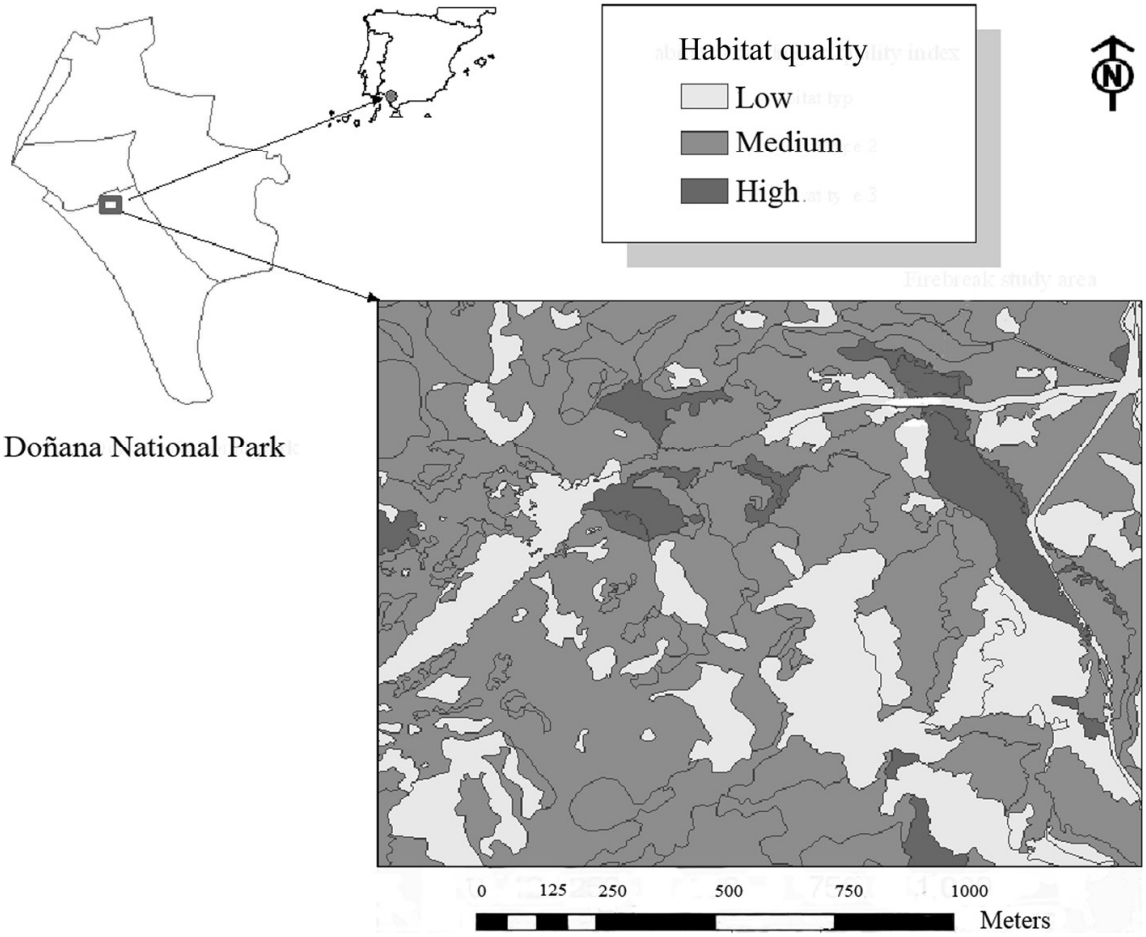

Fig. 1 Location of the study area within the Doñana National Park, SW Spain. The detailed map shows the boundaries of patches of eight original habitat types, and how they were reclassified into three classes according to habitat quality

Erica scoparia, *E. australis*). Elevated parts are dominated by xerophilous species, especially *Halimium halimifolium*, but also *H. conmutatum*, *Rosmarinus officinalis* and *Lavandula stoechas*.

Model Species

In Doñana National Park, the univoltine Lycaenid *P. argus* is by far the most abundant butterfly species, being on the wing during June (Fernández Haeger et al. 1976; Rodríguez 1991). During this period, the most abundant species providing nectar are the low shrubs *Thymus mastichina*, and *L. stoechas*, and the herb *Echium gaditanum*. *P. argus* depends upon both its larval host plant *H. halimifolium* and its mutualistic ant *Lasius niger* (Rodríguez et al. 1991, 1994). Almost all *L. niger* nests are located at the bases of *H. halimifolium* shrubs, because roots provide suitable sites for nest-building in the sandy soils (Jordano et al. 1992). The larval host plant is very abundant but butterfly occurrence tends to be restricted to areas with high density of *L. niger* nests (Rodríguez et al. 1994; Gutiérrez et al. 2005). Therefore, habitat quality for *P. argus* varies greatly with differences in the availability of host plants, mate density, nectar source plants, and mutualist ants (Jordano et al. 1992; Rodríguez et al. 1994). Accordingly, *P. argus* density greatly varies across the landscape (2–250 individuals per 100 m^2 plots 50 m apart; Gutiérrez et al. 2004).

Habitat Quality

We examined high-resolution orthophotographs (scale 1:5000; Junta de Andalucía 2004) with ArcMap 9.3 (ESRI 2011) to outline patches that we interpreted as homogenous, regarding structure, cover and identity of dominant plant species. Using these criteria, we identified eight different vegetation units. In order to quantify butterfly abundance and major components of habitat quality, we overlaid a 25-m grid on the resulting digital map. We used Hawth's Tools 3.27 (Beyer 2004) to draw a spatial random set of 111 cells in this grid. The distribution of cells across vegetation units was firebreak/pathway (2), bare dune (10), dune with 2–20 % shrub cover (17), dune with 25–50 % shrub cover (25), dense mixed scrubland containing the host plant (17), heathland (8), grassland and reed beds (19), and xerophilous scrubland with thyme *T. mastichina* (13).

We slowly walked a transect of 30 × 2 m along the diagonal of each cell and estimated *P. argus* abundance by counting flying individuals. We randomly selected a sample of up to 15 *H. halimifolium* individuals and dug the ground around their base or shook the stems to provoke the response of any ants and record whether a *L. niger* nest was present (Gutiérrez et al. 2004, 2005). We counted all nectar plants within a 1-m radius of sampled *H. halimifolium* shrubs. We used a cluster analysis with the Ward's method (van Tongeren 1995) to assess similarity between the eight classes of habitat quality on the basis of the quantitative indices obtained along the transects. The analysis revealed three distinct classes of vegetation units differing in habitat quality (Fig. 2). The lowest quality was found in either quite dense vegetation (heathland) or sparse vegetation units. Intermediate quality corresponded with grasslands, vegetated dunes

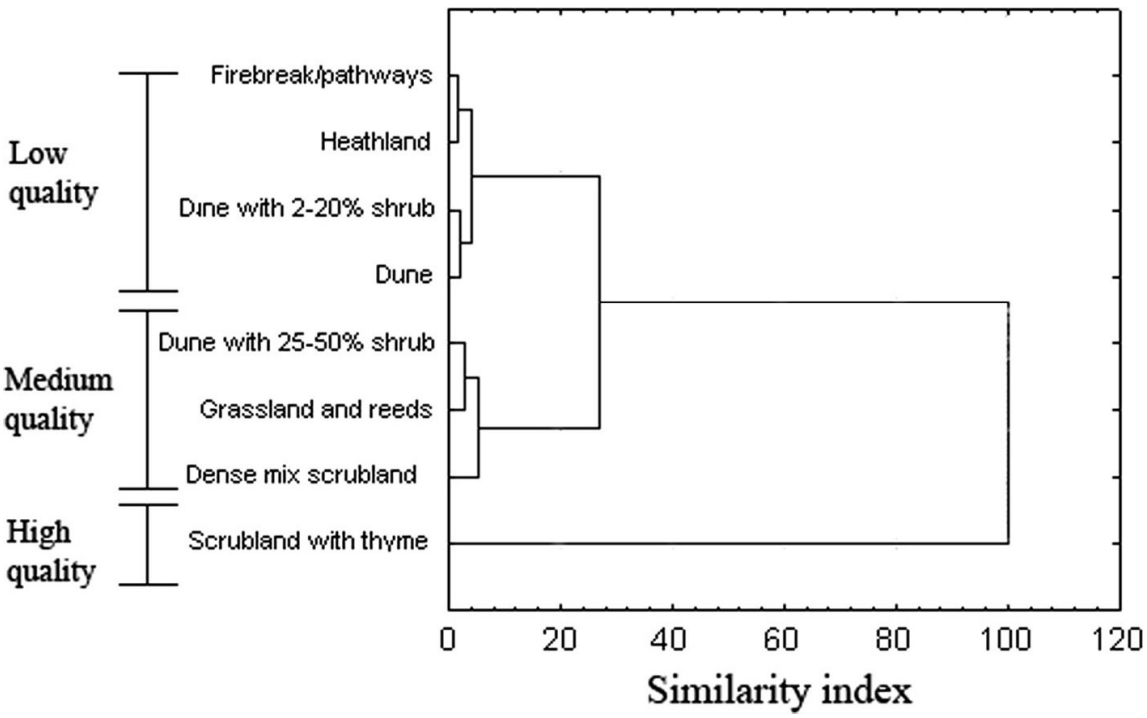

Fig. 2 Cluster analysis (Ward's method; van Tongeren 1995) of eight habitat types as a function of the number of butterflies, the density of host plants, the frequency of ant nests at the base of host plants, and the abundance of plant providing nectar around host plants. Similarity was determined using the ratio between the linkage distances for a particular case (D_{link}) divided by the maximum linkage distance (D_{max}), multiplied by 100

and dense mixed scrubland. The highest quality class only contained xerophilous scrubland dominated by *H. halimifolium* and also rich in thyme. Functional habitats resulting from the cluster analysis might represent better how *P. argus* perceive spatial variation in resource availability. Grouping patches of similar functional value resulted in a landscape less heterogeneous than the one we obtained by interpreting structural and floristic criteria (Fig.1).

Butterfly Movement

During 9–23 June 2008 and 13–27 June 2009, we used high-resolution tracks recorded by a handheld GPS unit (60X, Garmin, Schaffhausen, Switzerland) to recreate butterfly flying trajectories. Previously, we tested the accuracy and reliability of positions recorded by the GPS receiver when carried by an observer in motion. We repeatedly walked along the straight edges of a firebreak and found a nearly perfect match between the succession of points recorded by the GPS unit and the placement of the firebreak edge on high-resolution orthophotos. In addition, we selected isolated shrubs and other point features that were easily recognisable on orthophotos. We took one or more stationary positions per point to quantify error measurements of the GPS unit and calibrate butterfly positions. We found a mean (±SD) error of 0.033 ± 0.029 m (n = 331), with a maximum error of 0.25 m.

Considering the high density of *P. argus* and their short lifespan (Lewis et al. 1997 estimate a longevity of 3–4 days), we deem unlikely that a single individual were selected for observation more than once. Therefore, we consider our observation as independent samples representative of the butterfly behaviour in this population. We chose a flying individual at random and followed it keeping a distance of about 1 m, moving the receiver so that the trajectory was reproduced as accurately as possible. The GPS unit was set to acquire one position every second. This procedure did not cause any disturbance on butterfly behaviour that was evident to the observer. When the animal landed we recorded the position and duration of every butterfly stop. Exploratory movements of less than 25 cm within the same shrub were considered as stops and the observer remained stationary. Tracking ended either when we lost visual contact with the butterfly, when the butterfly began a prolonged stop (>5 min), or when we decided to end a long observation event after a shorter stop.

Successive positions were connected with linear segments named “steps”, and we measured the length of each step as well as the length of the whole path (the sum of step lengths). Net displacement was defined as the Euclidean distance between the first and last points of the path. We recorded the turning angle between adjacent steps, and computed its cosine in radians. Positive cosine values indicate forward progress, with a value of 1 meaning a straight forward path, while negative values indicate backward returns. We also calculated r, a measure of angular dispersion of observations ranging from uniform dispersion (r = 0) to complete concentration of observations in a single angle (r = 1; Batschelet 1981). As the time elapsed between successive positions was constant (the GPS unit took one position per second), step length also represents the speed of butterflies. As a measure of sinuosity of the flying paths, we use the ratio between path length and net displacement. Values close to 1 corresponded to highly directional flying paths, while larger values indicate an increase in sinuosity. The periods during which butterflies flew continuously, defined by two consecutive stops,

were named "sections". For whole paths and sections, mean step length and mean turning angle were calculated. We made all measurement in ArcMap 9.3, except for mean values of turning angle cosines (Oriana 4, Kovach Computing Services, Pentraeth, UK).

Autocorrelation

A pair of successive steps often shows similar values in speed or direction, especially at the high temporal resolution we used, making steps non-independent sampling units over which average parameter values can be computed to characterize butterfly trajectories (Swihart and Slade 1985; Turchin et al. 1991). To address this problem we calculated autocorrelation functions (ACFs) and autocorrelation values at different time lag windows of i = 1, 2, …, 30 s, that is, the correlation between the attributes of step x and those of step x + i. We considered autocorrelation to occur at values ≥0.6, so that the smallest lag with a value below that threshold indicated autocorrelation-free movement. For trajectories and sections within trajectories, we used the time lag for which autocorrelation blurred to redefine the beginning and end of steps, then we recalculated speed, sinuosity and turning angles.

Although autocorrelation may be an issue when steps are used as sampling units, it is also an attribute of trajectories conveying valuable behavioural meaning. We analyzed whether autocorrelation in step length varies with sex or the habitat across which the butterfly moved. We analyzed these effects in separate Generalized Linear Mixed Models (GLMM) where time lag was specified as a covariate, and individual identity as a random factor.

Detailed vs. Simplified Trajectories

We compared the detailed GPS trajectories with those that would have been obtained using a conventional method in which simplified paths were constructed with segments by linking points where butterflies were stationary, for example previously marked with flags. To construct the simulated simplified paths, we removed bouts of actual flight and recalculated movement parameters on the resulting path. Likewise, we built simplified versions of trajectories corrected for autocorrelation. Therefore, we obtained values of length, speed (when different from length), sinuosity, and turning angles on three versions of every trajectory: a) original GPS data, b) autocorrelation-free data, and c) simplified paths assuming that only butterfly stops were recorded (referred to as "conventional method"). We computed movement parameters for the complete path and for each section between consecutive stops. We used ANOVA to compare speed and sinuosity across methods, and circular statistics (Mardia-Watson-Wheeler test; Zar 1984) to compare turning angles.

Using paths within sections that were completely included in one of the three habitat quality classes, we examined the effect of sex and habitat quality on butterfly speed with a Generalized Linear Mixed Model (GLMM), specifying normal errors and individual identity as a random factor. We performed this analysis only on autocorrelation-corrected sections.

Behaviour at the Edge Between Patches of Different Habitat Quality

Butterfly decisions at the edge of two habitats with different quality were analyzed in a random sample of 100 flights among those recorded in at least two habitats. Although more complex responses to edges have been reported (Dover and Fry 2001), we considered four possible behaviours from the combination of two binary variables: whether or not the butterfly crossed the edge, and whether or not the butterfly explored or scanned the edge before making a decision. When a butterfly crossed an edge between two habitats, it could enter a habitat of better quality, a habitat of poorer quality, or a habitat of similar quality. We assigned a value of zero if there was no change in the quality of the two habitats across the edge, a value of 1 if the quality of habitat increased in one level (−1 in case of decrease) and a value of 2 if the increase was of two levels (−2 in case of decrease). We then obtained five categories of contrast in quality between adjacent habitats ranging from −2 to 2. We recorded the frequencies with which butterflies decided to cross edges as a function of the contrast in habitat quality at edge sides.

Results

Xerophilous scrubland was the sole vegetation unit identified as a high quality habitat for *P. argus*. Consistently, in xerophilous scrubland the percentage of host plants surrounded by nectar sources, number of shrubs providing nectar, butterfly density, and percentage of host plants sheltering *L. niger* nests were all much higher than in any other class of habitat quality (Table 1).

Autocorrelation

As expected, mean autocorrelation values for step lengths had an exponential distribution with high values in the first lags, and a quick decline for autocorrelation between steps recorded at more distant times within the path (Fig. 3). Mean autocorrelation of step lengths was below 0.6 at a time lag ≥4 s (Fig. 3a), so we took one every four

Table 1 Top: Comparison of mean (±SD) availability of host plants, nectar sources, mutualist ants, and *P. argus* density along 30-m random transects across three classes of habitat quality

	Habitat quality			F_{2109}	p
	Low (n = 37)	Medium (n = 61)	High (n = 13)		
Percent of host plants with ant nests	0.06 ± 0.13	0.31 ± 0.29	0.51 ± 0.24	12.819	<0.001
Percent of host plants with plants providing nectar within a 1-m radius	0.02 ± 0.07	0.05 ± 0.13	0.48 ± 0.47	5.814	0.004
Number of plants providing nectar within 1 m of host plants	0.19 ± 0.81	1.15 ± 3.08	25.0 ± 22.88	10.536	<0.001
Density of flying *P. argus*	2.86 ± 4.39	9.75 ± 9.89	39.0 ± 22.85	5.823	0.004

consecutive steps to build independent autocorrelation-free trajectories. Once the effects of lag and individual were accounted for, the model did not show significant differences between sexes in the autocorrelation of step lengths ($F_{1,498} = 0.42$, $p = 0.500$; Fig. 3a), so butterflies of both sexes were pooled in analyses of trajectories corrected for autocorrelation. Autocorrelation functions for step length had significantly different slopes for habitats of different quality (Fig. 3b; $F_{2,86} = 35.7$, $p < 0.001$), especially for time lags >3. In low-quality habitats, mean autocorrelation for step length decreases with increasing time lag at a slower rate than mean autocorrelation values in high-quality habitats (Fig. 3b).

Butterfly Movements

After excluding flying paths shorter than 10 m, we analyzed 565 trajectories containing 124,740 positions. The longest path length was 686.4 m, and this path was completed in 26.5 min (36.3 min if stops were included) with a maximum net displacement of 259 m. The mean (± SD) path length was 99.9 ± 89.0 m. The frequency distribution of recorded distances is shown in Fig. 4a. Over half individuals flew distances shorter than 50 m, but 199 butterflies were followed along paths longer than 100 m. The mean net displacement was 57.3 ± 52.0 m, with a maximum value of 343.7 m.

The mean speed per step was 0.45 ± 0.52 m/s, and the maximum speed recorded was 4.59 m/s. The frequency distribution of speed values indicates that 88 % records were <1 m/s (Fig. 4b). We recorded a total of 3160 stops which also included the initial and end points of trajectories. Stops defined 1528 sections, but we used for analysis only 1403 sections longer than 10 m. We recorded a maximum of 22 sections per trajectory. Ninety percent of turning angles were <45°, often <10° regardless of habitat quality. Only 3 % of turning angles were >90° and most were recorded in high quality habitats.

Comparison of Detailed, Autocorrelation-Corrected and Simplified Trajectories

Considering complete trajectories as sampling units, mean speed of original paths obtained by GPS tracking was only slightly higher than that in autocorrelation-

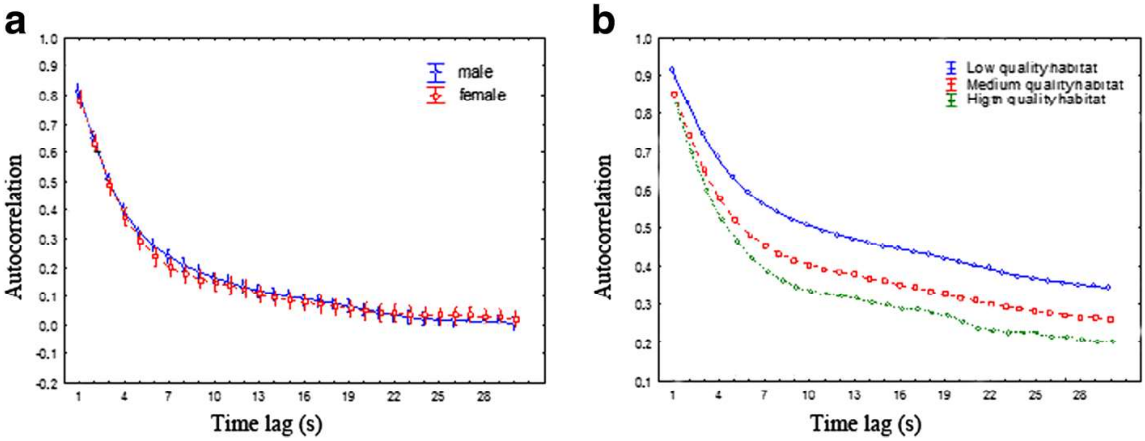

Fig. 3 Sexual (**a**) and habitat quality (**b**) differences in mean (± SD) autocorrelation of step length in complete trajectories. Time lags indicate the time elapsed between one-second steps used to resample the trajectory. For example, a time lag of 4 s indicates autocorrelation of one-second step lengths recorded four seconds apart. Whiskers were removed in panel B for the sake of clarity

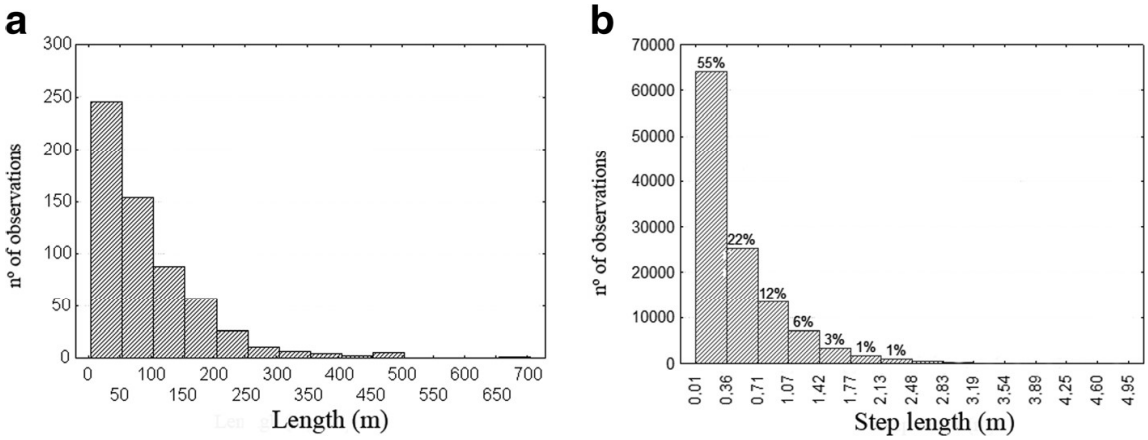

Fig. 4 **a** Frequency distribution of the length (m) of 565 complete paths. **b** Frequency distribution of step length (m), i.e. the distance travelled per second; n = 124,740

corrected trajectories (Fig. 5a). However, these mean values were about 50 % higher than mean speed of simplified trajectories ($F_{2,1693}$ = 18.295, p < 0.001 Fig. 5a). Likewise, mean values of sinuosity in original and autocorrelation-corrected trajectories were approximately 10 times higher than in simplified trajectories ($F_{2,1692}$ = 62.927, p < 0.001; Fig. 5c). The comparison of turning angles across the three ways of depicting trajectories yielded similar results. The mean cosine in original GPS trajectories (0.99 ± 0.01) was almost identical to the mean cosine of corrected trajectories (0.99 ± 0.01), but more than twice the mean cosine calculated in the simplified paths of

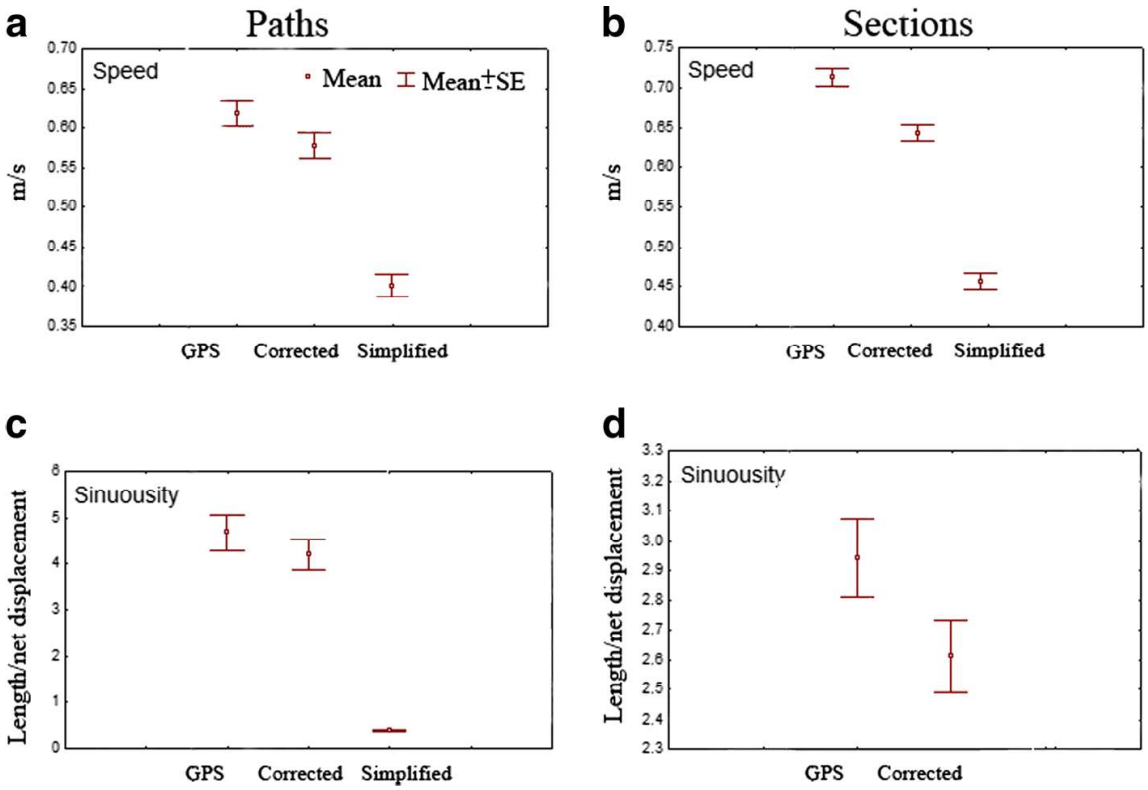

Fig. 5 Mean and standard error in speed and sinuosity calculated in complete paths (**a** and **c**) or path sections (**b** and **d**), as a function of the method used to define trajectories. GPS: original GPS paths; Corrected: autocorrelation-corrected paths where new steps are created by merging four consecutive steps in original GPS paths; Simplified: trajectories made by steps joining points where butterflies stopped

the conventional method (0.41 ± 0.66) which also showed considerable variance. These differences were significant ($F_{2,1514} = 166.86$, $p < 0.001$).

A similar pattern emerged when considering sections within trajectories as sampling units. Mean speed and mean sinuosity in original sections were higher than in sections corrected for autocorrelation, but these differences were not large (Fig. 5b and d). However, for butterfly speed, these means were 40–55 % higher than the mean in sections measured with the conventional method ($F_{2,4580} = 51.421$, $p < 001$). Compared with complete trajectories, mean speed in sections increased while mean sinuosity decreased (Fig. 5) as a trivial consequence of removing periods during which butterflies remained stationary. The mean cosine of turning angles in original sections (0.99 ± 0.01) was higher than in corrected sections (0.89 ± 0.20), and differences increased markedly when compared with the mean value in simplified sections obtained with the conventional method (0.23 ± 0.54; $F_{2,4573} = 439.61$, $p < 001$).

Mean turning angles between two consecutive steps were often very small in both original GPS paths (0.49°) and autocorrelation-corrected paths (1.01°). However, angular concentration in original trajectories ($r = 0.761$) was larger than in corrected trajectories ($r = 0.500$; Fig. 6). Greater deviation from straight movements was evident for simplified trajectories (mean turning angle =351.18°) which also showed larger variances ($r = 0.280$; Fig. 6).

Effect of Habitat on Movement Attributes

We recorded 1403 sections longer than 10 m: 493 in low quality habitat, 592 in medium quality habitat, and 89 in high quality habitat. For the analysis of habitat differences in movement attributes we used 1174 sections after excluding paths overlapping two or more habitat classes. As predicted, mean values of speed and section length increased significantly from high quality habitats to low quality habitats, whereas a significant decrease was observed for path sinuosity (Table 2). These significant differences were consistent across all three methods used to construct the trajectories (Fig. 7). The strong effect of habitat quality on speed was corroborated in a GLMM ($F_{2328} = 28.84$, $p < 0.001$) whereas the effect of sex was not significant ($F_{1353} = 2.85$, $p = 0.09$).

Likewise, in low quality habitat, mean turning angle (0.16°) was lower, and angular concentration higher ($r = 0.518$), than in medium quality habitat (2.05°; $r = 0.491$) and high quality habitat (358.14°; $r = 0.475$). Differences in mean turning angle between

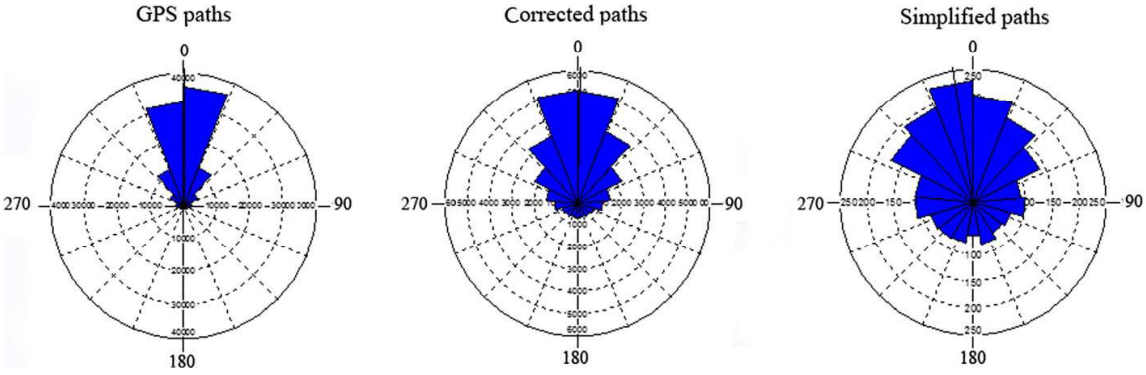

Fig. 6 Frequency distribution of turning angles between successive steps as a function of the method used to define trajectories (see Fig. 5)

Table 2 Comparison of mean (±SD) step length and sinuosity for complete trajectories, and path length for sections within trajectories, across three classes of habitat quality. It is also shown the F value and the significant level of ANOVA test

	Habitat quality			F	p
	Low	Medium	High		
Step length	0.45 ± 0.55	0.38 ± 0.4	0.36 ± 0.35	8768.5	<0.001
n	25,684	34,461	5390		
Sinuosity	2.82 ± 6.62	3.12 ± 4.55	3.55 ± 3.85	77.83	<0.001
n	528	667	95		
Section length (m)	30.60 ± 25.77	28.45 ± 24.99	23.32 ± 15.85	301.79	<0.001
n	528	667	95		

medium and high quality habitats were not significant (Mardia-Watson-Wheeler test, W = 3.15, p = 0.207). In contrast, we found significant differences in mean turning angle between low and medium quality habitats (W = 30.83, $p < 0.001$), and in mean turning angle between low and high quality habitats (W = 8.51, $p < 0.014$).

Behaviour at the Edge Between Patches of Different Habitat Quality

For paths longer than 100 m, individual butterflies remained in the same habitat in 51 % of trajectories, otherwise they crossed edges between habitats of different qualities at least once. Among the four possible decisions butterflies could make at habitat edges, the most frequent one (44 %) was crossing the boundary without any previous scanning or exploratory behaviour, whereas the less frequent decision (11 %) was to stay in the same patch. In 27 % of cases, butterflies refused to cross after scanning the boundary, while in 19 % of cases they scanned the habitat edge and eventually crossed it. We found differences in the propensity of butterflies to cross depending on the relative quality of the original and the approached habitat types ($\chi^2 = 34.5$, df = 12, $p < 0.001$; Fig. 8). We distinguished three different patterns. First, when edge crossing would entail a net gain of one or two levels in habitat quality, butterflies decided to cross without previous scanning with a frequency higher than expected, while they stayed in the patch of lower quality less than expected (Fig. 8d, e). Second, when individuals

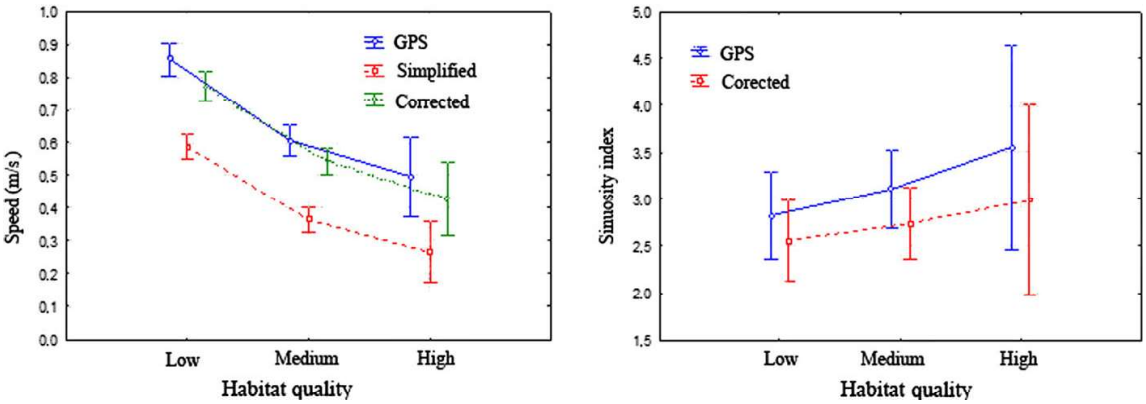

Fig. 7 Mean (± SD) of speed and sinuosity in sections across habitats of different quality

approached an edge between habitats of similar quality (no gain, Fig. 8c) or when crossing would imply a moderate loss of one level in habitat quality (Fig. 8b), they stayed more than expected and crossed less than expected. Finally, when the decision of crossing the edge would bring a marked loss in habitat quality, the behaviour observed much more often than expected was crossing after obtaining information at the boundary, whereas all other behaviours occurred less than expected (Fig. 8a).

Discussion

Assessment of GPS Tracking

Standard methods used to characterize butterfly movement (Dover and Fry 2001; Ries and Debinski 2001; Seymour et al. 2003; Korösi et al. 2008; Skórka et al. 2013) yield simplified representations of actual trajectories. These approaches may not reveal patterns in the response of butterflies to fine-grained distribution of important resources. Ecological mechanisms behind these behavioural patterns may be studied by measuring movements with higher spatial resolution (Cooke et al. 2004; Getz and Saltz 2008).

Only a few studies have used GPS devices to increase the precision of tracking butterfly flight paths. Some authors used GPS just to improve the spatial accuracy of the position of capture sites, or flags and other marks, dropped during conventional tracking (e.g. Weyer and Schmitt 2013; Kallioniemi et al. 2014). Others used tracks following butterflies but did not report its spatio-temporal resolution (Cormont et al. 2011) or set a temporal resolution of 15–20 s (Severns and Breed 2014). We show that high-resolution GPS tracking, at one point per second, is a feasible method to reproduce butterfly trajectories under some circumstances. Our study area is located in an open landscape on flat terrain which favoured butterfly detection, allowed keeping them in sight for long periods, and eased the movements of observers. More importantly, the absence of topographical accidents and tall vegetation allowed a very good satellite reception that helped us to achieve highly precise GPS tracks. *P.argus* individuals were easy to follow as they flew at speeds <1 m/s nearly 90 % of steps; lower feasibility is expected for species flying faster in habitats with denser vegetation. In optimal conditions, the reliability of GPS tracks may be only limited by the technical quality of handheld GPS receivers and the surveyor's ability to identify, follow and imitate the flight path. Finally, GPS tracking is inexpensive and offers an alternative to invasive procedures requiring the attachment of devices to the insect's body, such as telemetry or harmonic radar. As a result, the behaviour of a large number of individuals can be studied during the sometimes narrow temporal window of imago emergence.

Differences Between Trajectory Types

We compared movement attributes in raw GPS tracks at one-second resolution, slightly simplified tracks at four-second resolution where a part of autocorrelation between adjacent steps was removed, and highly simplified trajectories defined by straight line segments linking butterfly landing sites. We found that a loss of only 4 s in the temporal resolution of tracks did not qualitatively alter the pattern of *P. argus* movement across

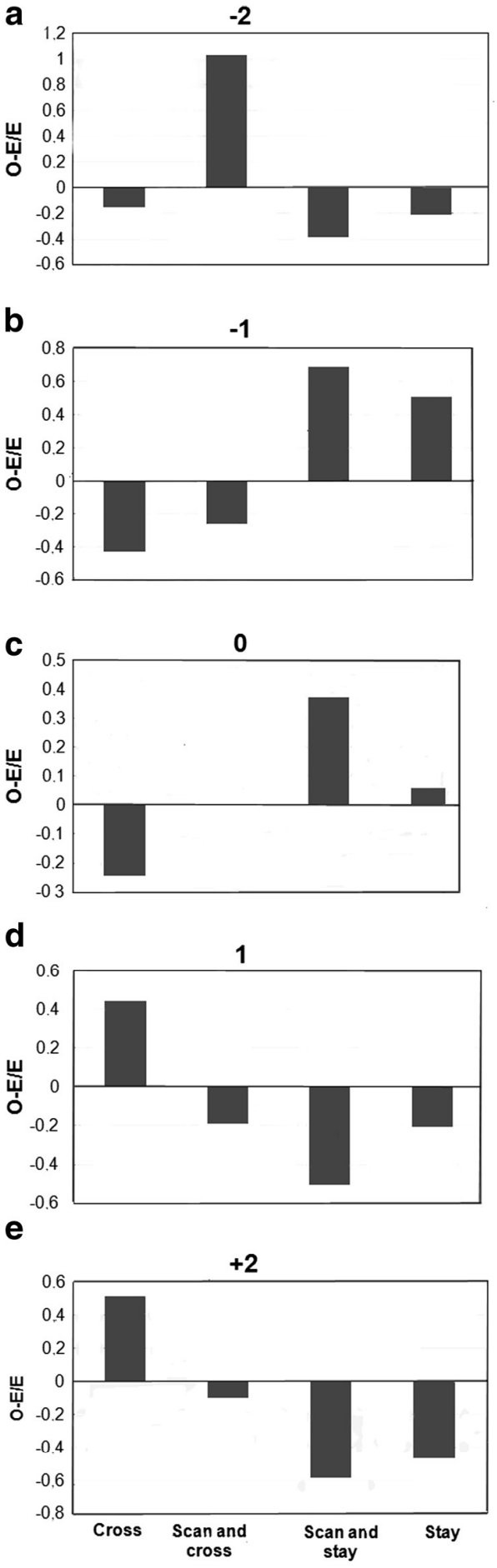

Fig. 8 Contribution to the chi-square statistic (O-E/E; O: observed frequency; E: expected frequencies) of frequencies of butterfly decisions at the edge of habitats with different quality. Three levels of habitat quality are considered, and changes in levels associated to edge crossing are indicated at the top of the panels as positive numbers when crossing leads to gains in habitat quality (e.g., +1: one-level improvement) or negative values when crossing involves loss in habitat quality

habitats of different quality. Differences in movement attributes between slightly simplified paths and original trajectories were generally small. However, even such a small loss of temporal resolution significantly reduced the estimated length of sections and the flight speed. We could also confirm and quantify the expected marked underestimation of length, speed, and sinuosity by oversimplified flying trajectories where nodes were defined by landing sites. Likewise, extreme simplification blurred the general pattern of generally straight forward progression by increasing the variance of turning angles. In simplified trajectories, reduction of mean cosine values suggests frequent changes in direction that are not real. Moreover, strong directionality at one-second resolution paths would appear incompatible with highly sinuous movements, especially in favourable habitats. Discrepancies between movement directionality in trajectories built with different temporal resolution may be reconciled if butterflies change direction gradually by the accumulation of many slight consecutive turns. Misleading effects of trajectory simplification were consistent in both complete paths and sections within specific habitats.

Recording behaviour at the highest spatial and temporal resolutions allowed by available techniques is desirable (Rooney et al. 1998). Most regular behavioural observations recorded on a limited subset of individuals, over a short period and in a restricted area, are autocorrelated (Otis and White 1999; Boyce et al. 2010). Autocorrelation of behaviour is not a problem in itself unless samples are too small or strictly independent data are required for the question addressed (Swihart and Slade 1997). Indeed, detailed accounts of a specific behaviour (e.g., how long it lasts or how often it repeats) are highly informative because they describe either persistently repeated or shifting instantaneous decisions. Moreover, detailed movement data such as those we collected on *P. argus* are relevant, for example, to better understand the costs (energy expense of flying over highly tortuous paths) and benefits (rewards in terms of nectar or host plants free from competitors) of flying patterns.

Movements in Habitat of Different Quality

Our results support the hypothesis that habitat quality determines the patterns of movement in *P. argus*. Butterflies had a fast-moving behaviour in habitats where the possibility of finding resources (nectar, egg laying substrate, or mates) was low, but they adopted a slower movement within resource-rich habitats. Winding movements in high quality habitats may reflect the need of acquiring detailed information before selecting a plant for egg laying, choosing a mate, or feeding on a mosaic of nectar sources depleted to different degrees. A shorter length of flights between consecutive stops in favourable habitats is consistent with the low effort needed to find abundant resources. Returns or sudden changes of direction were rare and, as they were recorded in high quality habitats, might be compatible with refusing to cross a boundary towards a lower quality habitat.

From an exploratory behaviour in high quality habitats, butterflies quickly shifted to more directional movements when flying across less suitable habitats (higher speed, smaller turning angles, less sinuous paths and longer steps). As reported in other species (Matter and Roland 2002), this suggests that *P. argus* may use cues to readily discern habitat quality.

Behaviour at patch edges further support the ability of *P. argus* to quickly detect changes in habitat quality without any longer exploration. Individuals seldom invested time and/or energy in scanning the boundary when they encountered a better habitat, suggesting that cues of an improvement in ecological conditions were acquired in advance (Schäpers et al. 2015) or, alternatively, that butterflies could have visited that patch before and were able to navigate back to it (Hawkes 2009). However, in agreement with other studies (Schultz et al. 2012), encountering patches of similar or slightly lower quality elicited boundary scanning, usually before refusing to cross. Butterflies may trace loops around edges, exploring the habitat in a non-random movement (Conradt et al. 2000; Conradt and Roper 2006). If valuable resources could be detected from distant points, the need of acquiring information at boundaries less contrasted in habitat quality suggests that cues may function better with marked differences in resource density. Finally, scanning also preceded edge crossing towards patches of the lowest quality. Interestingly, very few butterflies stayed in the original patch after approaching such contrasted edges. This behaviour suggests an intentional travel across unsuitable habitat, perhaps with the aim of reaching a different high quality patch that was previously visited or detected because it was within the butterfly perceptual range (Merckx and van Dyck 2007). In the case of females, escaping for male harassment (Severns and Breed 2014) in crowded patches, competition (high female density), or associated depletion of suitable spots for egg laying within shrubs containing nests of mutualistic ants would predict a quick cross without previous scanning. Decision-making of crossing the edge or staying in the current patch may be strongly influenced by the expected net gain of habitat quality.

An increasing number of studies show that movement of animals is not a random process, and that resource distribution determines movement patterns across heterogeneous habitats (Conradt et al. 2000; Berggren et al. 2002; Revilla et al. 2004; Söderström and Hedblom 2007). The relative contribution of structural and functional elements in the landscape as determinants of animal movement is still poorly understood (Schultz et al. 2012). We conclude that patterns of movement in *P. argus* were explained by spatial heterogeneity of the fundamental resources that butterflies need to survive and reproduce. Conversely, movements were not so well predicted by habitat patches defined solely by structural elements such as scrubland density. Therefore, this species responds to spatial heterogeneity in habitat quality described on the basis of functional criteria.

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[CAPÍTULO 7]

"BEHAVIOR OF THE BUTTERFLY PLEBEJUS ARGUS FACING A FIREBREAK IN A MEDITERRANEAN LANDSCAPE"

“BEHAVIOR OF THE BUTTERFLY *PLEBEJUS ARGUS* FACING A FIREBREAK IN A MEDITERRANEAN LANDSCAPE”

Resumen

Contexto

Los cortafuegos son tiras lineales que diseccionan el paisaje y previenen o mitigan la propagación de incendios forestales en los paisajes mediterráneos. Sin embargo, pocos estudios han abordado su posible efecto sobre el comportamiento animal. La falta de tráfico y otras actividades humanas en los cortafuegos los hacen adecuados para probar el único efecto de la interrupción del hábitat físico en el movimiento de los animales.

Objetivos

Nuestro principal objetivo fue evaluar si el patrón de movimiento de una especie de mariposa se vio afectado por este elemento del paisaje.

Métodos

Reconstruimos las trayectorias de vuelo de la mariposa lycénida *Plebejus argus* dentro y alrededor de un cortafuegos utilizando el rastreo visual y GPS en el Parque Nacional de Doñana (sur de España).

Resultados

Las mariposas que estaban activas en el límite del cortafuegos a menudo se negaron a entrar y, cuando lo hicieron, la mayoría de los individuos regresaron antes de llegar al lado opuesto. Dentro del cortafuegos, registramos trayectorias más rápidas y rectas que en áreas de matorral adyacentes. Las mariposas que cruzaron el cortafuegos se dirigieron hacia la dirección más favorable para minimizar el tiempo que pasaron dentro de la discontinuidad del hábitat. A escala del paisaje, la densidad del cortafuegos aumentó en las áreas donde el hábitat de *P. argus* estaba más fragmentado y tenía menor calidad.

Conclusiones

Un elemento lineal estrecho y abierto que carece de actividad humana induce un cambio notable en el comportamiento del movimiento de una especie de mariposa, con consecuencias potenciales en la dinámica de la población a escala del paisaje. Por lo tanto, los cortafuegos utilizados para proteger los paisajes mediterráneos podrían tener efectos secundarios en poblaciones animales distintas de la pérdida de hábitat localizada debido a la mera eliminación de vegetación.

Behavior of the butterfly *Plebejus argus* facing a firebreak in a Mediterranean landscape

Pilar Fernández [1], Alejandro Rodríguez [2], David Gutiérrez[3], Diego Jordano[1] & Juan Fernández Haeger[1]

[1]Department of Botany, Ecology and Plant physiology. University of Córdoba. Campus de Rabanales. Edificio C-4. 14071 - Córdoba, Spain.

[2]Department of Conservation Biology, Estación Biológica de Doñana-CSIC, Américo Vespucio 26, 41092 Sevilla, Spain

[3]Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, 28933 Móstoles, Madrid, Spain

Abstract

Context

Firebreaks are linear strips that dissect the landscape and prevent or mitigate the spread of wildfires in Mediterranean landscapes. However, few studies have addressed their potential effect on animal behavior. The lack of traffic and other human activities in firebreaks makes them suitable for testing the sole effect of physical habitat disruption on animal movement.

Objectives

Our main objective was to evaluate whether the pattern of movement by a butterfly species was affected by this landscape element.

Methods

We reconstructed flight trajectories of the lycaenid butterfly *Plebejus argus* within and around one firebreak using visual and GPS tracking in Doñana National Park (southern Spain).

Results

Butterflies that were active at the firebreak boundary often refused to enter the firebreak and, when they did, most individuals returned before reaching the opposite side. Inside the firebreak we recorded faster and straighter trajectories than in adjacent scrubland areas. Butterflies that crossed the firebreak headed the most favorable direction to minimize the time spent within the habitat discontinuity. At the landscape scale, firebreak density increased in areas where *P. argus* habitat was more fragmented and had lower quality.

Conclusions

A narrow, open linear element lacking any human activity induces a marked change in the movement behavior of a butterfly species, with potential consequences on population dynamics at the landscape scale. Therefore, firebreaks used for protecting Mediterranean landscapes could have side effects on animal populations other than localized habitat loss due to mere vegetation removal.

Keywords: firebreaks, animal movement, butterflies, edges, behavior

Introduction

The viability of animal populations in fragmented landscapes ultimately depends on the ability of individuals to reach and settle in patches of favorable habitat (Thomas and Harrison 1992; Bowler and Benton 2005; Morales et al. 2010). This ability will be greatly influenced by the compound probability of animal transfer between a series of adjacent habitats of different quality (Aben et al. 2012). The interaction between habitat structure and individual decision making determines the movement of species across the landscape (Merckx et al. 2003) which, in turn, reflects responses to the presence or absence of resources along its trajectory (Pereira and Rodríguez 2010; Bergerot et al. 2012; Eycott et al. 2012; Loos et al. 2015).

It is important to estimate the relative suitability of habitat patches for exploiting resources as well as determining the permeability of supposedly inhospitable matrix to animal movement (Eycott et al. 2012). Along this gradient of friction to movement, the presence of relatively hard edges may be inferred from behavioral patterns both at an initial stage (i.e. when animals approach or encounter the habitat boundary; Fernández et al. 2016), and during transitions through relatively hostile patches (Schtickzelle et al. 2007). Mechanisms by which matrix may inhibit movement include a higher mortality risk (Revilla and Wiegand 2008; Shepard et al. 2008; Polic et al. 2014; Keret et al. 2015), increased energetic cost due to physiological stress (Baker and Rao 2004), physical resistance of some matrix types (Eycott et al. 2012) and lack of resources, such as food and shelter (Haynes et al. 2007).

Numerous studies have shown the importance of the boundary between two habitats in the decisions animals make about where to move. Boundaries may be diffuse, with a gradual discontinuity between suitable and unsuitable habitat or may appear as abrupt discontinuities between adjacent patches of contrasted quality (Collinge and Palmer 2002; Haynes and Cronin 2006). Structural discontinuities that are evident to the human eye may or may not be perceived as such by animals (van Dyck 2012); some encourage their movement while others

prevent or hinder it. These divergent responses are species-specific (Ries and Debinski 2001; Suárez-Esteban et al. 2013), and may vary within species (Morales et al. 2010).

Linear elements may reduce or interrupt animal dispersal (Munguira and Thomas 1992; Shepard et al. 2008; Polic et al. 2014; Keret et al. 2015), may be neutral (Leidner and Haddad 2010) or even may facilitate movement (Dover and Fry 2001). It is difficult to establish to which extent this reduced demographic flow across the matrix can be attributed to increased mortality or merely to a marked reluctance of animals to visit patches that are poor in resources. Firebreaks are relatively wide linear structures without vegetation cover, which create an abrupt change in resource availability and abiotic conditions in the forest landscapes they help to protect. Firebreaks are free from human activity (apart from clearing) and, consequently, from associated mortality risk typical of structurally similar landscape elements such as roads (e.g. exposure to toxic chemicals, traffic casualties during flight or when attracted to the warm tarmac). On the other hand, existing studies on firebreaks focus on their effect on species diversity where firebreaks are simply considered as grass patches (Kubo et al. 2009; Ohwaki et al. 2018), or as a source of edge effects involving pollination and seed dispersal (Suárez-Esteban et al. 2013 and 2014) with interesting opposite effects for different animal groups (Suárez-Esteban et al 2013). We find, however, a lack of studies on the effect of these structures on insect movement patterns.

In this study, we analyze the behavior of the lycaenid butterfly *Plebejus argus* when encountering the abrupt structural discontinuity of a cleared firebreak dissecting high quality breeding habitat in Doñana National Park (DNP). We selected this species because it has been the focus for numerous studies on distribution and dispersal in different parts of its range (e.g., Thomas and Harrison 1992; Lewis et al 1997; Kubo et al. 2009). In addition, its distribution is well known in the study area, where it occurs in large numbers, and it is easily tracked with the handheld GPS units (Fernandez et al. 2016). We assume that butterfly mortality risk from natural causes (e.g. predation) does not increase substantially when they use the firebreak for

a short period. However, food, shelter, and other essential resources are absent in the barren ground and we hypothesize that the firebreak will be avoided or seldom used. For individuals entering the firebreak, we also hypothesize that the marked change in ecological conditions will alter butterfly behavior and therefore flight attributes. More precisely, we firstly predict that, when encountering the firebreak boundary, individuals will tend to avoid entering this hostile environment, and we expect high rates of refusal. Secondly, we predict that once individuals enter the firebreak they should fly in a way that minimizes the time spent in this apparently unsuitable habitat. Therefore, inside firebreaks, we expect faster flights and more rectilinear trajectories than in the surrounding scrubland. We also expect butterflies to select the path direction leading to leave the firebreak as soon as possible.

Methods

Study area

Our research was carried out in DNP, which is located on a large area of sandy soils along the Atlantic coast in southwestern Spain (Fig. 1). In this flat area, firebreaks contribute significantly to a dense network of barren linear strips that also include paved and unpaved roads allowing vehicle traffic. This network covers over 2190 km over an area of 545 km^2 (density of 4.02 km^{-2} of which 777 km (35%) are firebreaks (Román et al. 2010; Suárez-Esteban et al. 2014). Firebreaks are maintained yearly by mechanical processes (plowing) giving rise to 20 to 30-m wide bands deprived of grasses or shrubs. We chose an area of sparse shrubs up to 1-m high where *Halimium halimifolium* (the main host plant of *P. argus*) is the dominant species, and scrubland is dissected by a 20-m wide firebreak (Fig. 2a). This firebreak runs in east-west direction and is representative of other firebreaks in DNP.

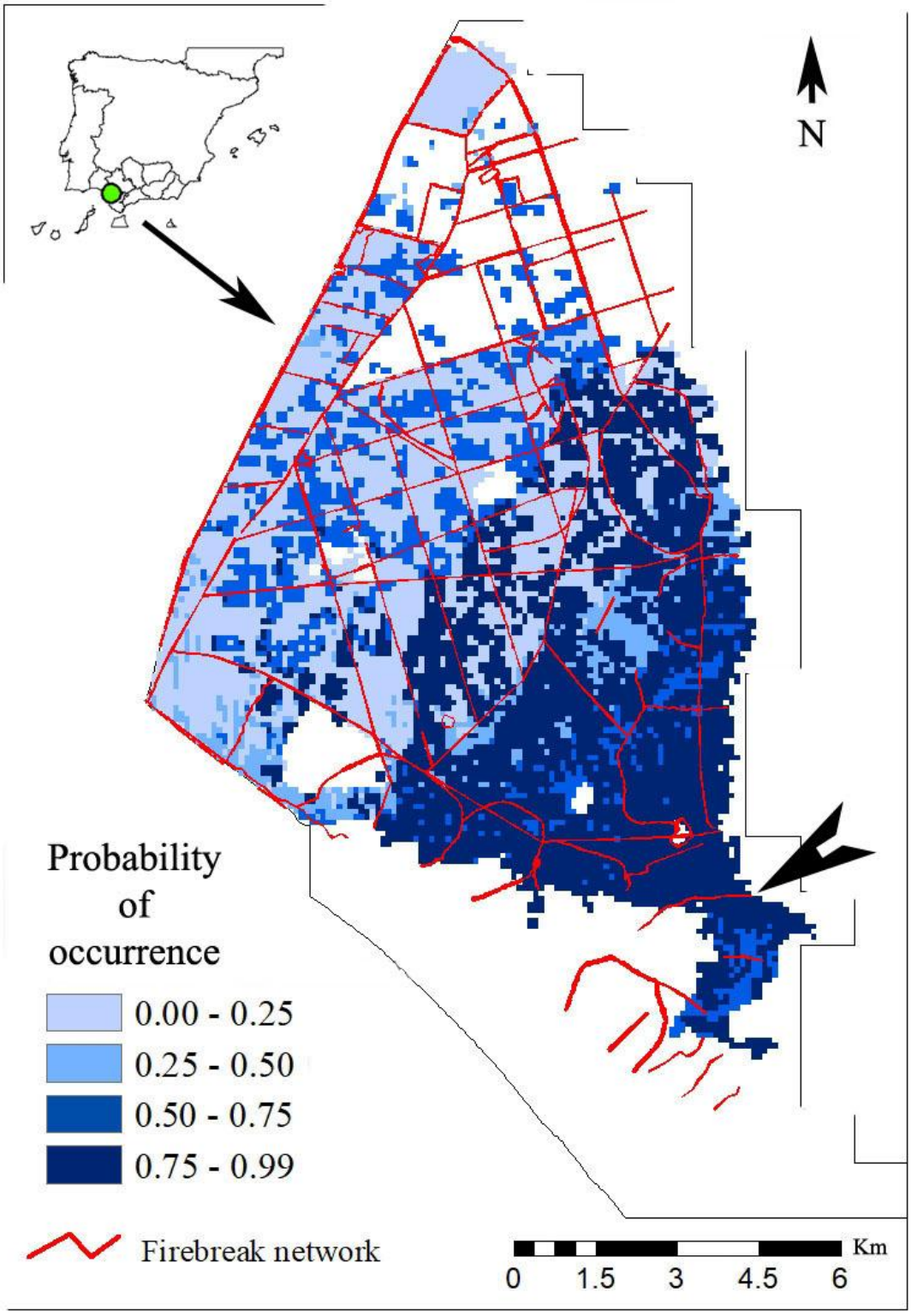

Fig. 1 Probability of occurrence of *P. argus* in 100-m cells within *H. halimifolium* scrubland of the Doñana National Park, as predicted by the habitat model by Gutiérrez et al. (2005). Location of the study area within Doñana National Park (SW Spain) is also shown.

Model species

The silver-studded blue *P.argus* is a lycaenid butterfly widely distributed in Europe and Asia. The species is common in the northern half of the Iberian Peninsula but has a highly fragmented distribution further south (Fernández et al. 2015). The southernmost isolated population in Europe occurs in DNP, where this butterfly is univoltine, being on the wing between mid-May and the first week of July, while reproduction usually concentrates during June (Rodríguez et al. 1991). Larvae depend on the presence of the host plant *H. halimifolium* and maintain an obligate mutualism with *Lasius niger* ants (Jordano et al. 1992). In this area, the density of *P. argus* adults shows substantial variation at small spatial scales, ranging from 2 to 250 individuals 100 m^{-2} in sites only 50 m apart (Fernández et al. 2017).

Butterfly behavior at the firebreak boundary

Field observations of adults were carried out in an area of medium to high butterfly density on two days, 12 and 18 June 2008, between 9:00 and 15:30, i.e. during the period of highest butterfly activity. Fieldwork was done on warm days (mean hourly temperature = 26.7°C, range: 23.9-30.2°C) under light winds (mean hourly wind speed = 4.0 ms^{-1}, range: 2.2-6.5 ms^{-1}, further details of climate variables in Online Resource 1, Fig. S1). Butterfly density was estimated by counting individuals in flight along two linear transects (50-m long by 2-m wide) across scrubland adjacent to the firebreak that were compared with the counts in other two transects placed in nearby habitats of lower quality (see Fernandez et al. 2016). We estimated a density of 40 ± 2 butterflies 100 m^{-2} on 12 June, and 14 ± 6 butterflies 100 m^{-2} on 18 June in scrubland adjacent to the firebreak. These values were much higher than those recorded in the transects across lower quality habitat, 9 ± 3 butterflies 100 m^{-2} on 11 June, and 3 ± 3 butterflies 100 m^{-2} on 18 June. The estimate for each day was the mean for the two transects in each habitat.

Up to three observers walked slowly in opposite directions along the boundaries of a 700-m section of the study firebreak. Observers randomly chose an individual flying within 1 m of the boundary (either inside or outside the firebreak), noted its sex, observed its trajectory, and assigned the observed behavior to one of the following crossing categories (Fig. 2b):

No attempt.- The butterfly left the 1-m boundary strip towards scrubland or stayed within the strip after a 2-min observation period;

Less than halfway.- The focal animal entered the firebreak but turned back before reaching half its width;

More than halfway.- The animal flew beyond half the firebreak width but turned back to the original scrubland area without reaching the opposite firebreak boundary; or

Crossing.- The butterfly crossed the firebreak and entered the scrubland at the opposite side.

Observations of butterflies entering the firebreak ended once they contacted the scrubland again. Observers kept walking at least 5 m and then haphazardly chose another flying individual.

Although butterflies were not captured or marked there is strong indication that the probability of tracking the same individual two or more times was very low. First, the probability of choosing different animals in different observations increases with butterfly density, and density values were high. Second, as the mean lifetime of adults is 4 days (Smith 2000), very few individuals observed on 12 June might have survived to be recorded six days later. Finally, a low probability of choosing the same animal twice is expected if butterfly spontaneous movements are short relative to the speed of the observer. Adults of *P. argus* are relatively sedentary. In a random sample of 30 flights lasting 1 min, the mean (±SD) net distance covered (11.1 ± 7.2 m) was less than half the approximate distances walked per minute by searching observers (24 m).

The frequency of flights in different crossing categories (Fig. 2b) and the effect of sex were analyzed with contingency tables and chi-square tests. We analyzed the frequency with which (a) butterflies left the scrubland to enter the firebreak, (no attempt *vs* attempt paths), (b) butterflies entered the firebreak and returned after attaining different depths into the firebreak (less *vs* more than halfway paths), and (c) butterflies left the scrubland and reached the other side of the firebreak (returning paths *vs* crossing paths).

Movement patterns

During the 2008 and 2009 *P. argus* flying seasons, we used GPS tracking to record butterfly movements (Fernandez et al. 2016). Three experimented observers equipped with a handheld GPS unit (Garmin 60 CX) followed butterflies approximately at a distance of 1 m to minimize disturbance and recorded their position at 1-s intervals. Butterflies often land on plants for feeding, resting or ovipositing. We recorded the coordinates of landing sites that were called stops. Tracking ended either when the individual was lost or when butterfly rested on a shrub for at least 5 min; therefore, stops lasted <5 min. We found that individuals that rested that long were likely to remain immobile for much longer, and the 5-min cut-off allowed us to maximize the number of individuals that could be tracked.

Butterfly positions along their flying trajectory were plotted and analyzed using ArcMap 9.3 (ESRI 2008) (Fig. 2b). For each trajectory, we defined paths, steps and sections (Fig. 2c). Path was the whole sequence of positions of the trajectory recorded for each individual. Step was the line connecting two consecutive points taken at 1-s intervals along the flight trajectory. Since each path may cross different habitats, we subdivided each path into segments completely included in one of three possible habitat types: scrubland, at least 2 m off the firebreak limit; boundary, within 2 m of the firebreak boundary, inside or outside; and firebreak, at least 2 m inside the firebreak. These were called habitat segments. As segment lengths were highly variable, we standardized flights by randomly choosing five samples of 10

consecutive steps (sections henceforth) per habitat (Fig. 2c). Absence of stops was a condition for drawing these random samples.

For paths, we computed the following movement metrics. Length is the total distance travelled (m); net displacement is the Euclidean distance (m) between starting and ending points; speed equals length divided by flying time (s); net speed is net displacement divided by flying time; sinuosity is defined as the ratio between length and net displacement; directionality denotes the ratio between the longest and the shortest axes of the minimum convex polygon encompassing all positions of a path. Directionality is highest for straight trajectories, whereas for lasting exploratory movements its value is close to 1, corresponding to a polygon approaching a circular shape.

We compared the attributes of paths that eventually crossed the firebreak with those of unsuccessful attempts. We used linear models (LMs) including length, net displacement, speed, net speed, sinuosity and directionality as response variables, and crossing success, number of stops and their interaction as explanatory variables. We included the number of stops as an additional variable in models because they may affect movement patterns at least in two ways: (1) following resting, individuals might cover longer distances or travel at higher speed; (2) searching for and settling on a suitable perch may result in bypaths altering sinuosity and directionality. Length, net displacement, speed, net speed and sinuosity were log-transformed to normalise residuals. Using maximum likelihood (ML), we fitted all possible models considering different combinations of fixed variables. We considered the best model as that with the smallest Akaike Information Criterion corrected for small sample size (AIC_c) (Burnham and Anderson 2002). To determine the truly most parsimonious model, we selected (1) those models with ΔAIC_c (AIC_c difference with the best model) values <6; and (2) within this set, we retained only those models which did not have simpler, higher-ranking variants, i.e. including a smaller number of the same explanatory variables (Richards et al. 2011).

To examine the effect of habitat and sex on movement patterns, we considered the attributes of sections and steps. As duration was constant in both cases, length and net displacement were equivalent to speed and net speed, respectively. Section attributes were speed, net speed, sinuosity and directionality, and step attributes were step length, turning angle and azimuth. Turning angle (degrees) was the angle between two successive steps, while azimuth was the azimuth of the line connecting two successive points.

We used linear mixed-effects models (LMMs) with section and step attributes as response variables, habitat and sex as fixed effects, and individual as random effect. Speed, net speed and directionality were log-transformed, sinuosity was cosine transformed, while turning angle and azimuth were cosine transformed, then rescaled to 0-1 interval and arc-sine square root transformed for normalising residuals (Turchin 1998).

For LMMs, we first assessed the inclusion of individual identity as a random effect following Zuur et al. (2009). For each response variable, we fitted models with restricted maximum likelihood (REML) containing all fixed effects but with different random structure (one model with a random intercept of individual and another without any random effect). Because these models were nested with respect to their random component, we used a likelihood-ratio test to determine which model best explained the data (Bolker et al. 2009). Then, to select the best model for each response variable, we followed the same approach based on ML and AIC_c as for path attributes (see above, Burnham and Anderson 2002). Final models were refitted using REML and their adequacy was examined by plotting residuals against fitted values (Zuur et al. 2009).

For each sample of turning angles, we calculated the circular mean and the mean vector length (r) as a measure of dispersion in 16 circular arcs of equal length (Zar 1984). Turning angles were then classified in three categories: forward steps, corresponding to angles from 0º to 45º or from 315º to 360º; turning steps, indicative of a change in trajectory with angles from 225º

to 315º or from 45º to 135º; and backward steps, including angles from 135º to 225º. As the firebreak was east-west oriented, crossing minimizing flying time would follow a north-south direction. Azimuths were also classified into two categories: trajectories approximately perpendicular to the firebreak, towards either north or south, including azimuth values in the same range as turning angles of forward or backward steps defined above; and trajectories approximately parallel to the firebreak, with other azimuth values. We used contingency tables and chi-square tests to compare differences in the distribution of turning angles and azimuths across habitat types and sexes.

Analyses were performed with R (R Development Core Team 2015) using packages 'nlme' (Pinheiro et al. 2014) and 'MuMIn' (Bartoń 2016), Statistica (StatSoft Inc. 2007), and Oriana (Kovach Computing Services 2005) for circular statistics. All spatial analyses were made with ArcMap (ESRI 2008) and Hawth`s analysis tools (Beyer 2004).

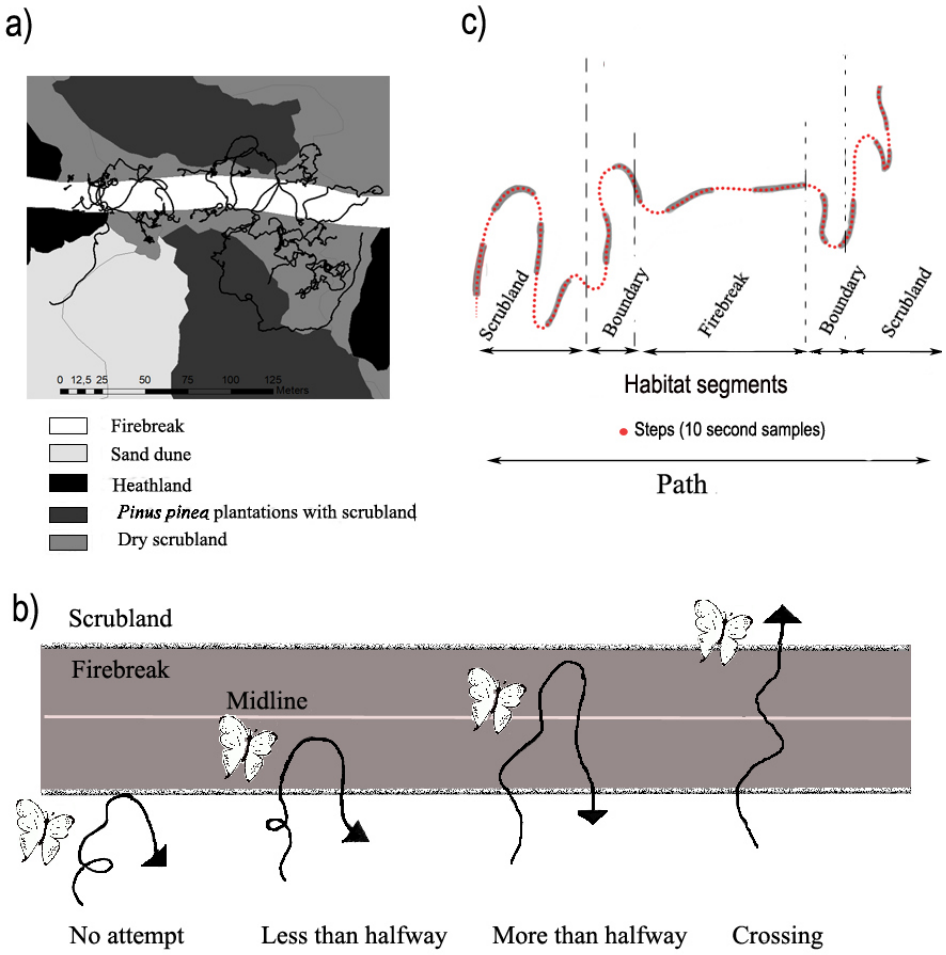

Fig. 2 a) Habitat map of the study area showing a stretch of firebreak (white band). Some butterfly tracks reconstructed with GPS are also shown. b) Schematic representation of categories of flight trajectories by butterflies first spotted within 2 m of the firebreak boundary (gray strip). In class 'No attempt', the butterfly left the 1-m boundary strip towards scrubland or stayed within the strip after a 2-min observation period. In class 'Less than halfway', the butterfly entered the firebreak but turned back before reaching half its width (white line). In class 'More than halfway', the butterfly flew beyond half the firebreak width but turned back without reaching the opposite boundary. In class 'Crossing', the butterfly crossed the firebreak and entered the scrubland at the opposite side. c) Schematic drawing of an exemplar path showing steps and sections at the three habitat types (scrubland, boundary and firebreak). Sections, i.e. 10-s long random samples along the butterfly trajectory, are marked as grey bands.

Spatial configuration of Doñana firebreak network relative to habitat quality

To evaluate their potential effects on the distribution of *P. argus*, we examined the density and spatial configuration of firebreaks relative to butterfly habitat quality. As a surrogate of habitat quality we used the probability of occurrence of *P. argus* based on previous large-scale research at 100-m grid resolution over a large area (92.36 km^2) of terrestrial habitats dominated by *H. halimifolium* scrubland within the DNP (Gutiérrez et al. 2005). We considered models for probability of occurrence instead of abundance of *P. argus* because the former was much more robust in evaluation tests. The model included three predictors, namely the expected probability of *L. niger* occurrence, presence of heathland (a surrogate of water availability), and slope (Gutiérrez et al. 2005). We used this model to create a map of predicted probability of occurrence for *P. argus* (Fig. 1). We classified the probability of occurrence into four categories: very low (0-0.25), low (0.25-0.5), high (0.5-0.75) and very high (0.75-1), following the categories used in the original model (Gutiérrez et al. 2005). We then calculated the distance from the center of each 100-m grid cell to the nearest firebreak, testing whether there were statistical differences between categories by means of analysis of variance and post-hoc Tukey tests (Unequal N HSD).

Results

Butterfly behavior at the firebreak boundary

We recorded the behavior of 526 butterflies, 227 females and 299 males (Table 1). Overall, 335 individuals (64%) did not leave the scrubland to enter the firebreak (Fig. 2b). No association was found between each sex and any of the four crossing categories (Table 1).

Only 191 (36%) butterflies entered the firebreak. The overall frequency of male visits to the firebreak (62%) tended to be higher than that of females (46%), but the effect did not attain formal significance (p=0.056, Table 1). For 191 butterflies entering the firebreak, 24% effectively crossed it, whereas 76% returned to scrubland, most of them (90%) before reaching

the midpoint. Only 15 individuals returned after passing the firebreak midpoint (Table 1). No significant differences between sexes were found for depth of flight into the firebreak and crossing success (Table 1).

Table 1 Frequency of crossing behaviors and movement decisions for male and female *P. argus* flying in scrubland and encountering a barren firebreak. Contingency table analysis tests the independence between crossing categories and sex (df=3), and between movement decisions and sex (df=1 in all cases).

Behavior		**Male**	**Female**	**Chi-square**	**p**
Crossing behavior					
No attempt paths		180	155	4.87	0.182
Less than halfway		85	46		
More than halfway		8	7		
Crossing success		28	19		
Movement decision					
Crossing attempts	No attempt	180	155	3.64	0.056
	Attempt	119	72		
Depth of flight into the firebreak	< Halfway	85	46	0.78	0.378
	> Halfway	8	7		
Crossing success	Return	93	53	0.51	0.474
	Crossing	26	19		

Movement patterns

Crossing *vs*. non-crossing paths

For the analysis of movement, we used flight data of 39 butterflies approaching the firebreak. Fourteen individuals (36%) crossed the firebreak (2 of them returned to the starting edge after entering the opposite scrubland), while 25 flew along the border or entered the firebreak but eventually returned to scrubland. Paths of individuals crossing the firebreak were longer, in terms of either length or net displacement, than paths of those that did not cross, but the effect was dependent on the number of stops (interaction between success and stops) (Table 2; Online Resource 1 Table S1). Using either speed or net speed, individuals crossing the firebreak moved faster than those that did not cross, with a negative effect of number of stops for net speed (Table 2). Sinuosity and directionality did not differ between individuals that crossed vs. those that did not cross the firebreak, but there was a positive effect of number of stops (Table 2).

Table 2 Parameter estimates of fixed effects (crossing, number of stops, and their interaction) for the best models of movement attributes of *P. argus* based on flight paths (n = 39). Crossing is a binomial variable (success or failure). The intercept includes de effect of level 'failure'

Variable	Parameter	SE	t	p
Length				
Intercept	1.07	0.08	12.786	<0.001
Success	0.73	0.16	4.680	<0.001
Stops	0.06	0.01	5.219	<0.001
Success*Stops	-0.03	0.02	-1.935	0.061
Net displacement				
Intercept	0.69	0.08	8.657	<0.001
Success	0.84	0.15	5.644	<0.001
Stops	0.03	0.01	3.055	0.004
Success*Stops	-0.04	0.02	-2.713	0.010
Speed				
Intercept	-0.64	0.07	-9.555	<0.001
Success	0.20	0.11	1.777	0.084
Net speed				
Intercept	-0.99	0.11	-9.306	<0.001
Success	0.254	0.16	1.581	0.123
Stops	-0.04	0.01	-3.048	0.0043
Sinuosity				

Intercept	0.33	0.05	7.093	<0.001
Stops	0.03	0.01	6.142	<0.001
Directionality				
Intercept	0.49	0.04	12.888	<0.001
Stops	0.02	0.00	3.669	<0.001

Effect of habitat type

We obtained 424 sections, 118 in scrubland, 238 at the firebreak boundary, and 68 inside the firebreak. Linear mixed models had a better fit than linear models not including butterfly identity as a random term for speed (likelihood-ratio=78.31, df=1, p<0.001), net speed (likelihood-ratio=62.97, df=1, p<0.001), sinuosity (likelihood-ratio=5.48, df=1, p=0.065), directionality (likelihood-ratio=8.44, df=1, p=0.015), step length (likelihood-ratio=916.08, df=1, p<0.001), turning angle (likelihood-ratio=39.35, df=1, p<0.001), and azimuth (likelihood-ratio=11.58, df=1, p=0.003). Mixed models including the main effects of habitat and sex were the most parsimonious for speed, net speed and step length, whereas best models for other movement parameters retained the effect of habitat only (Online Resource 1 Table S2). The speed and net speed of flights across the firebreak were higher than in the other habitats, and these differences were significant (Table 3). Speed and net speed in males were higher than in females and these differences were larger for sections recorded in the firebreak (Fig. 3).

For sinuosity and directionality, the most parsimonious model included only the effect of habitat. In the case of sinuosity, the null model was also in the confidence set (Table S2). Values of sinuosity and directionality in the firebreak were significantly lower than in scrubland or boundary (Table 3).

For the analysis of step length, turning angle and azimuth, our dataset contained 1292 observations in scrubland, 2717 in the boundary, and 753 in the firebreak. In the firebreak,

mean step length was significantly longer (Table 3, Fig. 3) than in other habitat types. Mean step length for males was higher than for females (Table 3, Fig. 3).

Table 3 Parameter estimates of fixed effects (habitat and sex) in selected mixed models of movement attributes of *P. argus* in flight sections within homogeneous habitat types. Butterfly identity was specified as a random factor. The intercept includes de effect of level scrubland (within factor habitat) and of level female (within factor sex). Attributes of sections (10-s random samples of butterfly flights) include speed, net speed, sinuosity and directionality index. Attributes of steps were length, turning angle and azimuth.

Variable	Parameter	SE	t	p
Attributes of sections				
Speed				
Intercept	-0.06	0.10	-0.61	0.544
Habitat (Boundary)	0.13	0.05	2.46	0.014
Habitat (Firebreak)	0.43	0.07	-6.1	<0.001
Sex (Male)	0.32	0.12	2.69	0.011
Net speed				
Intercept	-0.23	0.11	-2.15	0.033
Habitat (Boundary)	0.12	0.06	1.99	0.047
Habitat (Firebreak)	0.47	0.08	-5.80	<0.001
Sex (Male)	0.35	0.12	2.86	0.007
Sinuosity				
Intercept	0.72	0.01	51.42	<0.001
Habitat (Boundary)	-0.01	0.01	-0.42	0.676

Habitat (Firebreak)	-0.05	0.02	-2.51	0.0128
Directionality				
Intercept	0.142	0.01	20.05	<0.001
Habitat (Boundary)	-0.011	0.01	-1.57	0.117
Habitat (Firebreak)	-0.032	0.01	3.26	0.001
Sex (Male)				
Attributes of steps				
Step length				
Intercept	0.12	0.07	1.69	0.091
Habitat (Boundary)	012	0.01	8.17	<0.001
Habitat (Firebreak)	0.52	0.02	25.85	<0.001
Sex (Male)	0.21	0.09	2.38	0.023
Turning angle				
Intercept	0.33	0.01	28.28	<0.001
Habitat (Boundary)	-0.02	0.01	-1.49	0.1366
Habitat (Firebreak)	-0.11	0.01	-7.26	<0.001
Azimuth				
Intercept	0.64	0.02	28.32	<0.001
Habitat (Boundary)	0.02	0.02	1.58	0.1141
Habitat (Firebreak)	-0.14	0.02	-7.02	<0.001

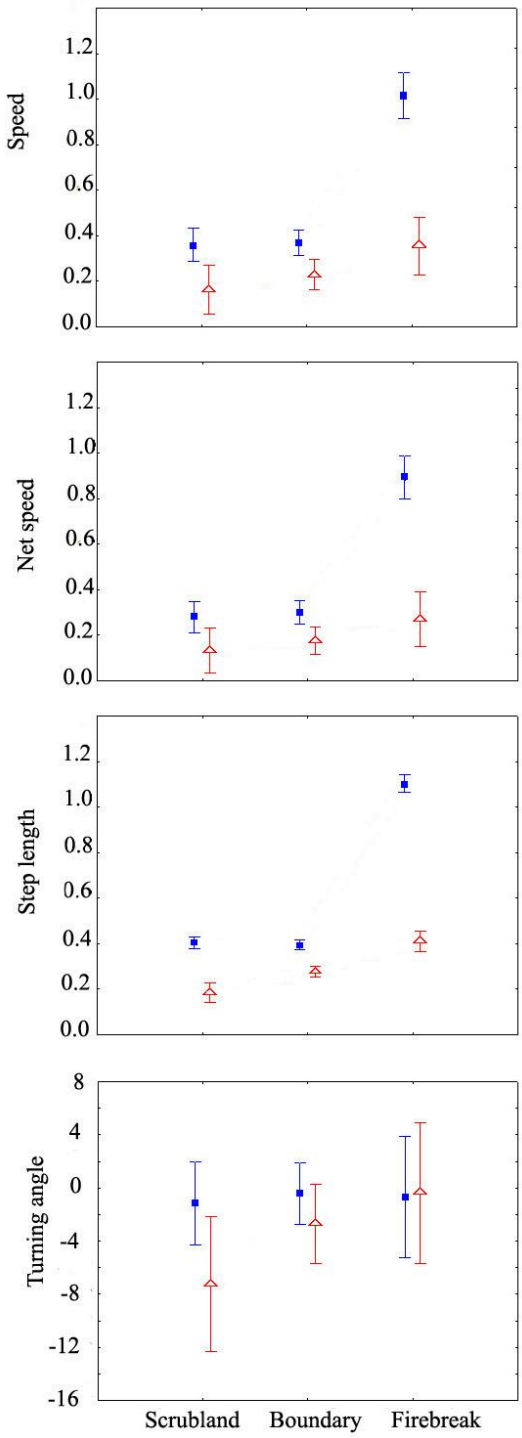

Fig. 3 Mean (±SE) speed (m s^{-1}), net speed (m s^{-1}), step length (m) and turning angle (degrees transformed) in path sections (standardized 10-s steps) of *P. argus* across scrubland (females, n= 37; males, n=81), firebreak (females n=26; males n=42), and the boundary between them (females, n=96; males n=142). Results shown for females (red triangles) and males (blue squares).

The most parsimonious model for turning angle included habitat as the only predictor (Table S2). Turning angles in firebreak sections were significantly smaller than in the other two habitat types (Table 3). Turning angle of flights in the firebreak (Fig. 4) had a mean vector μ=1.0º (r=0.872, n=740) that differed (χ^2=105.3, p<0.001) from mean vector in the boundary 359.3º (r=0.748, n=2663). Mean vector in scrubland (μ= 357.9º, r=0.711, n=1264) differed from the mean vector in both boundary (χ^2=6.041, p=0.049) and firebreak (χ^2=107.608, p<0.001). These statistical differences were marked but the effect size was very small as most consecutive steps were highly autocorrelated with a value of mean vector μ close to 0 and observations quite clustered around this mean in the three habitats considered (length of the mean vector r close to 1). The frequency distribution of turning angles based on three categories varied across habitat types, suggesting a dominance of backward steps in scrubland and forward steps in the firebreak (for further details, see Online Resource 1 Supplementary results and Fig. S2).

The most parsimonious model for azimuth included only habitat (Table S2), being firebreak sections significantly different from the other two habitats (Table 3). Butterflies in firebreak tended to fly mainly following north-south direction (μ=7.1, r=0.215, Fig. 4b), close to the shortest crossing distance for an east-west oriented structure (Fig. 2). In scrubland and boundary, we observed flights in all directions (Fig. 4d, f). At boundary, the mean azimuth pointed to the southwest (μ =236.6, r=0.066), approximately matching the direction of the firebreak edge. These differences across habitats were significant (χ^2=82.4, df=15, p<0.001). In the firebreak, the mean azimuth also differed significantly (χ^2=64.5, df=15, p<0.001) from the mean azimuth in scrubland (μ=283.6, r=0.128; Fig 4f). Mean azimuths in scrubland and boundary were also significantly different (χ^2=18.649 df=15 p<0.001). The frequency distribution of azimuths, simplified into two categories, was significantly different among habitats, with a dominance of flights perpendicular to the firebreak (Supplementary results and Fig. S2).

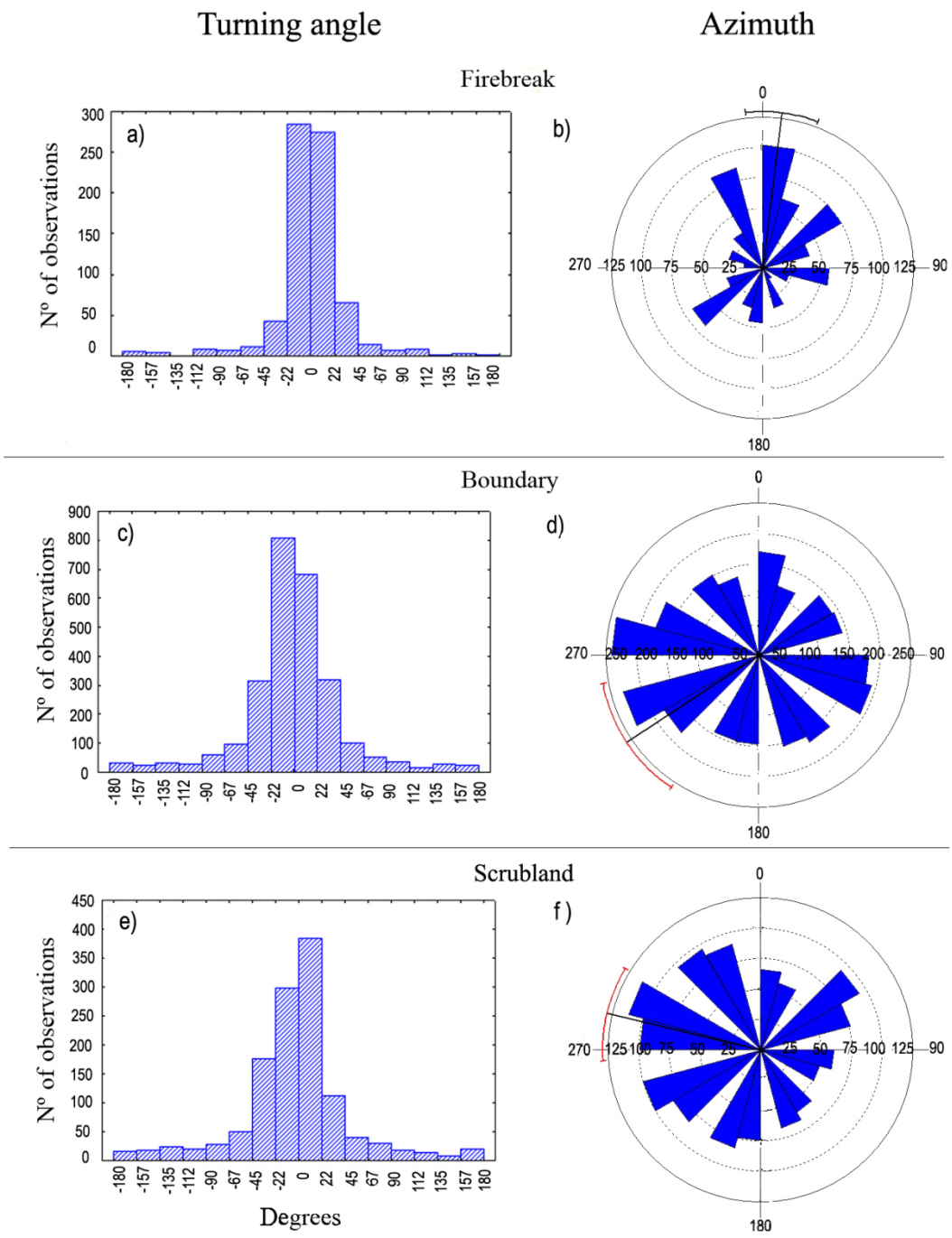

Fig. 4 Left panel: frequency of observations of turning angles (a, c, e) in the three habitat types (scrubland, boundary and firebreak). Fat tails in the distribution of turning angles indicate that trajectories in scrubland and boundary were more tortuous than in the firebreak. Right panel: frequency of observations of azimuth classes (using 22.5º intervals) (b, d, f), the mean of the distribution (radial line) and the dispersion around the mean (outer arc).

Distance from 100-m grid cells to the nearest firebreak significantly differed between classes of probability of occurrence ($F_{3, 9516}$=119.95, p<0.001; Fig. 1). The mean distance (± SE) to the nearest firebreak for grid cells with very low probability of occurrence of *P. argus* was shorter (176.5±3.2 m) than for grid cells with very high probability of occurrence (254.8±2.8 m). Mean distances to the nearest firebreak for grid cells with intermediate categories of probability of occurrence were intermediate: 205.4±7.6 m for grids with low probability, and 196±4.7 m for grids with high probability.

Discussion

Our results show that firebreaks somehow constrain the movements of *P. argus.* We observed not only marked changes in the patterns of movement inside the firebreak but also a clear behavioral response of butterflies encountering this linear structure. Most butterflies were reluctant to enter the firebreak and less than 10% of the butterflies that flew into it were able to cross its entire width. It is worth noting that firebreak width (20 m) was within the perceptual range of similar lycaenid butterflies (e.g., 10-22 m for *Icaricia icarioides*, Schultz and Crone 2001). If *P. argus* had a similar perceptual range, individuals could detect suitable habitats at the other side of the firebreak but, apparently, they rarely embarked into crossing attempts. Therefore, linear barren structures wider than the perceptual range of *P. argus* might exert a filter to movement stronger than the one we detected. Butterflies seem to perceive negatively such relatively narrow open space supporting the hypothesis that firebreaks or similar open linear structures without human activity act as a semipermeable barrier to movement. Although the study is based on a single firebreak, the observed patterns could be representative of many other firebreaks existing in the DNP, all with similar widths, same bare sand soil and identical management.

Seasonal fluctuations of flowering plants, location of their oviposition substrate, plant size, the need to maintain body temperature and the presence of mutualistic ants in some species are the main factors driving daily or seasonal movements to satisfy all needs of butterflies (Brommer and Fred 1999; Kubo et al. 2009, Fernández et al. 2016). In some species, the existence of adequate plant cover, in quantity and/or quality, determines how the species evaluates and uses space and the subsequent movement pattern (Eycott et al. 2012; Loos et al. 2015).

When firebreaks are mowed instead of ploughed, they may be beneficial for butterflies, especially when the resulting grassland is scarce in the landscape (e.g. Ohwaki et al. 2018). In contrast, in our system barren firebreaks do not provide any valuable resource for *P. argus* and its environmental conditions probably entail considerable physiological stress. No nectar or host plants are available. Butterflies cannot find perches to rest, or refuge to hide from predators. Firebreaks in the study area are maintained clean by systematic ploughing at the end of spring, so shade provided by vegetation is lacking and butterflies find no opportunity for behavioral thermoregulation in a quite hostile environment. This butterfly species is on the wing during the summer solstice when solar radiation is intense and bare sandy soils can reach temperatures up to 60ºC (Angulo et al. 2007). This high soil temperature could be lethal for the butterflies if they remain close to the ground for a long time (Mazer and Appel 2001). Kubo et al. (2009) reported that *P. argus* benefited from using a firebreak because resources, especially nectar, abounded. In our study firebreak, resources are absent during the mating season, supporting the idea of a resource boundary (Schultz et al. 2012) more than a structural boundary. The contrast between our findings and those by Kubo et al. (2009) illustrates how the same landscape structure can be perceived differently by the same organism depending on its resource content (Ries and Sisk 2004). In our study area, there is plenty of nectar and ovipositing plants at both sides of the firebreak but a complete absence of both resources inside it. Moreover, most individuals entering the firebreak did not cross it, despite the

effective crossing distance is clearly in the range of the flight distances described for the species (Gutiérrez et al. 2004; see also Schultz & Crone 2001). This suggests that individuals venturing into the barren space do not find cues for resources and opt for coming back to the scrubland.

The firebreak not only limits crossing attempts but also alter the way butterflies fly across it. As expected, flights were faster and more rectilinear compared with flights in suitable habitats of our study area, where the pattern of movement should be associated with foraging (Fernández et al. 2016), or other tasks (Schultz and Crone 2001; Haynes and Cronin 2006; Hawkes 2009). Movements inside the firebreak resemble the pattern described for butterfly displacement in contrast to routine movements (van Dyck and Baguette 2005). This behavioral response might help to shorten the time spent in an inhospitable space, and similar patterns have been reported in a variety of organisms (Turchin 1991; van Dyck and Baguette 2005; Fahrig 2007). The most effective way for the butterflies to minimize the time spent in the firebreak would be a perpendicular crossing. This was the choice of most individuals entering the firebreak, suggesting that they have information about the size and configuration of the discontinuity and/or about the presence of resources at the other side (Andrews and Gibbons 2005). It would be sensible considering memory and previous experiences in further analysis of movement (Bowler and Benton 2005; Clobert et al. 2009; Morales et al. 2010).

We found sex-specific differences in movement behavior. *P. argus* males move faster and straighter than females, and also tended to cross the firebreak more often than females. Males and females have been reported to behave differently when facing resource and structural boundaries (Bergerot et al. 2012; Schultz et al. 2012; Kallioniemi et al. 2014; Mair et al. 2015). Females may be, as a rule, more concentrated in offspring requirements and especially in finding sites suitable for oviposition (in our case, host plants with ants nests), whereas males explore habitat more broadly to attract or find mates (Dover and Rowlingson 2005; Schultz et al. 2012) and are supposed to be more prone to face the risk of an hostile scenario.

Firebreaks are not anecdotal features in Mediterranean landscapes. In DNP, the density of unpaved roads and firebreaks is so high than more than 80% of the area is less than 200 m from one of these linear structures (Román et al. 2010). In humanized landscapes, roads alter landscape spatial pattern and reduce or interrupt horizontal ecological flows (Skórda et al 2013; Muñoz et al 2015; Andersson et al. 2017). These landscape structures may act as barriers for dispersal by increasing mortality (Munguira and Thomas 1992; Shepard et al. 2008; Polic et al. 2014; Keret et al. 2015; Keilsohn et al 2018) or through active behavioral avoidance during dispersal movements. It is difficult to establish to which extent this reduced demographic flow across matrix can be attributed to increased mortality of merely to a marked reluctance of animals to visit patches poor in resources. The physical structure of roads is similar to that of the firebreak we studied, but traffic and diffuse pollution of roads might additionally reduce survival of individuals living nearby or attempting crossing. Therefore, we predict that the effects of roads (either unpaved or paved) on butterfly behavior would be even stronger than those described here.

Although dispersal is usually associated with faster and directional movements, it can also occur as a by-product of 'routine' movements for feeding, mating or resting, particularly in fairly continuous landscapes (van Dyck and Baguette 2005). If behaviour during dispersal entailed decisions as those observed here, firebreaks could interfere with an important demographic process and, therefore, could have negative consequences on the *P. argus* population of the study area. Those effects would be expected to be stronger in the northwest portion of DNP, where these linear developments are closer to butterfly habitat of lower quality and showing more fragmentation (Fig. 1). Nevertheless, to explore the potential demographic effects, we would have to additionally incorporate the effects of more subtle habitat components, as mutualistic ant density, which generates low contrast patch edges (to the human eye) for *P. argus* (Seymour et al 2003).

Our study was restricted to a single firebreak and a particular species. Large-scale studies evaluating firebreaks of different widths and landscape context, and also involving other species, are needed to generalize these results. Even so, our results show that a narrow and open linear element is able to generate a marked change in flight behavior, create a functional discontinuity in the landscape and, therefore, limit to some extent the movement of butterflies. Firebreaks are widely used to protect forested areas in Mediterranean ecosystems. Among other functional shortcomings, firebreak efficiency for stopping high-intensity fires in Mediterranean forests has been questioned (Plana et al. 2005) and here we concur with other authors (Suárez-Esteban et al. 2014) that they may also have ecological side effects other than the mere removal of vegetation. The impact of firebreaks on insect populations may depend on the combined effects of physical factors such as the width of the discontinuity, management (e.g. maintenance through livestock grazing; Ruiz-Mirazo et al. 2011), and species-specific traits such as mobility.

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Supplementary material for

Journal name: **Animal conservation**

Article title: **Behavior of the butterfly *Plebejus argus* facing a firebreak in a Mediterranean landscape**

Authors: Pilar Fernández [1], Alejandro Rodríguez [2], David Gutiérrez[3], Diego Jordano[1] & Juan Fernández Haeger[1]

[1]Department of Botany, Ecology and Plant physiology. University of Córdoba. Campus de Rabanales. Edificio C-4. 14071 - Córdoba. Spain.

[2]Department of Conservation Biology, Estación Biológica de Doñana-CSIC, Américo Vespucio 26, 41092 Sevilla, Spain

[3]Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, 28933 Móstoles, Madrid, Spain

Corresponding author: Pilar Fernández. E-mail: bv2ferop@uco.es

Online Resource 1

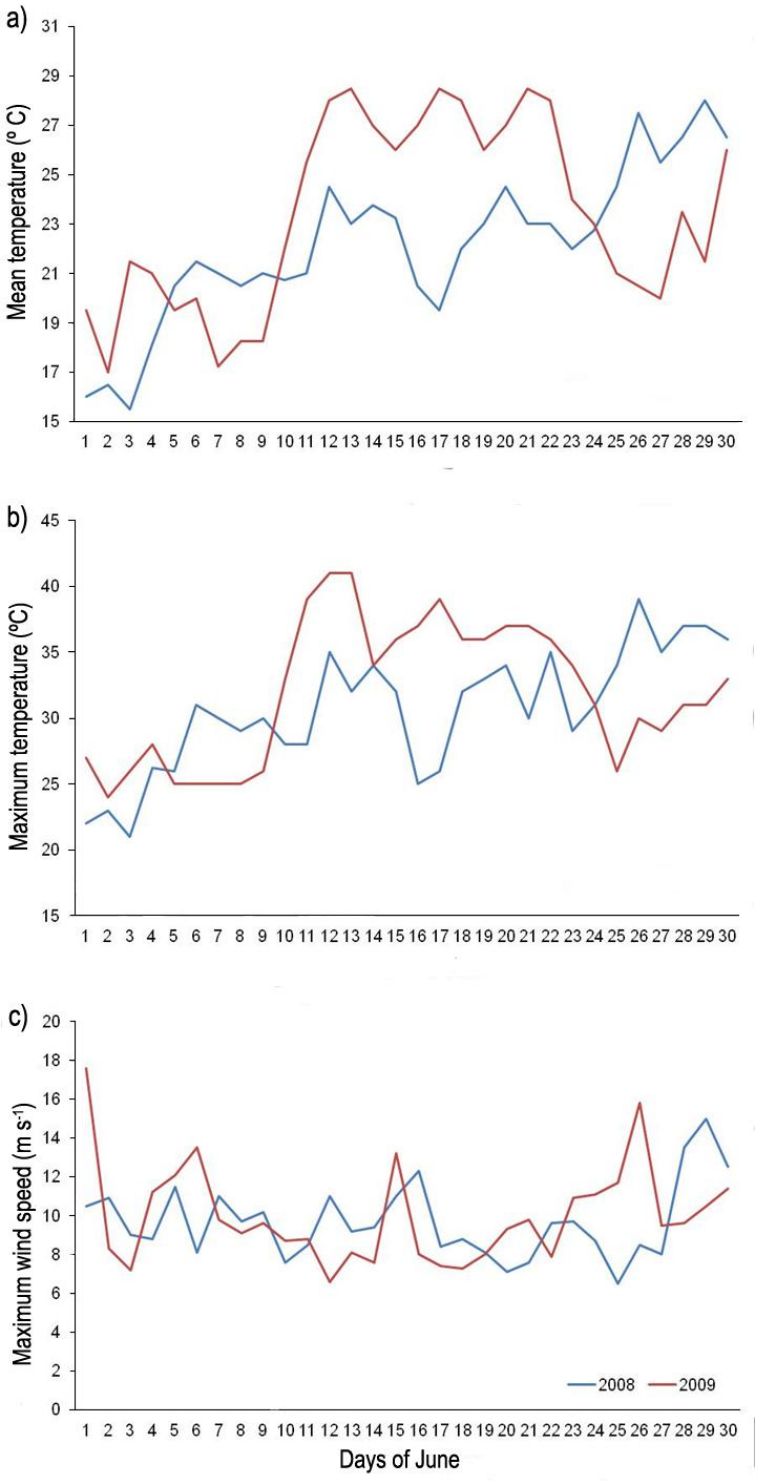

Fig. S1. Mean (a) and maximum (b) daily temperatures, and maximum wind speed (c) in June during 2008 (blue line) and 2009 (red line) in Doñana. Data from 'Palacio'(a, b) and 'Control RM1' (c) meteorological stations. Precipitation in June was zero in both study years (not plotted, data from 'Palacio' meteorological station). URL: http://icts.ebd.csic.es/es/datos-meteorologicos)

Table S1 Selection of linear models considering the effect of different combination of number of stops and crossing success (Success/failure) on movement variables of butterfly paths. Response variables were length, net displacement, speed, net speed, sinuosity and directionality. LL: log-likelihood; AIC_c: corrected Akaike's Information Criterion; ΔAIC_c: difference in AIC_c between current and best model

Variable	Model	LL	AIC_c	ΔAIC_c
Length	Success * Stops	-10.82	33.47	0.00
	Success + Stops	-12.81	34.79	1.32
Net displacement	Success * Stops	-8.97	29.75	0.00
	Success	-13.89	34.46	4.72
Speed	Success	-11.92	30.53	0.00
	null model	-13.52	31.37	0.84
Net speed	Success + Stops	-24.04	57.26	0.00
	Stops	-25.35	27.39	0.13
	null model	-28.81	61.96	4.69
Sinuosity	Stops	4.77	-2.85	0.00
Directionality	Stops	12.76	-18.83	0.00

Table S2 Selection of linear mixed models considering the effect of different combinations of sex and habitat on movement parameters of sections (i.e., 10-s butterfly flight samples) and steps. Individual identity was included as a random effect in all models. Response variables were speed, net speed, sinuosity, directionality index, step length, turning angle and azimuth. LL: log-likelihood; AIC_c: corrected Akaike's Information Criterion; ΔAIC_c: difference between current and best model

Variable	Model	LL	AIC_c	ΔAIC_c
Attributes of sections				
Speed	Habitat + Sex	-250.14	514.5	0.00
	Habitat	-253.68	519.6	5.10
Net speed	Habitat + Sex	-307.23	628.7	0.00
	Habitat	-311.18	634.6	5.80
Sinuosity	Habitat	270.28	-528.4	0.00
	null model	266.67	-525.3	3.11
Directionality	Habitat	589.309	-1166.4	0.00
Attributes of steps				
Step length	Habitat + Sex	-2199.9	4413.8	0.00
	Habitat	-2202.7	4417.4	3.60
Turning angle	Habitat	-1063.264	2138.5	0.00
Azimuth	Habitat	-2331.5	4675.0	0.00

Supplementary results

Movement patterns

Effect of habitat type

The frequency distribution of turning angles based on three categories varied across habitat types. In scrubland, forward steps occurred less than expected from a homogeneous distribution, whereas turning steps and especially backward steps were recorded more than expected (χ^2=11.01, df=2, p=0.004, Fig. S2a). At boundary, we found an approximately even distribution of steps among the three classes of turning angles (χ^2=1.61, df=2, p=0.448, Fig. S2a). However, in the firebreak, the frequencies of turning and backward steps were significantly lower than expected, whereas forward steps were observed more than expected (χ^2=45.14, df=2, p<0.001, Fig. S2a).

When azimuth was classified into two classes, we found statistically significant differences in the distribution of flight directions among habitats (Fig. S2b). In scrubland, the frequencies of steps running parallel or perpendicular to the firebreak were similar (χ^2=0.10, df=1, p=0.755). At boundary, the frequency of parallel steps was higher than that of perpendicular steps (χ^2=7.12, df=1, p=0.008; Fig S2b). In contrast, in the firebreak, the frequency of steps perpendicular to its main axis was much higher than that of parallel steps (χ^2=29.91, df=1, p<0.001; Fig. S2b).

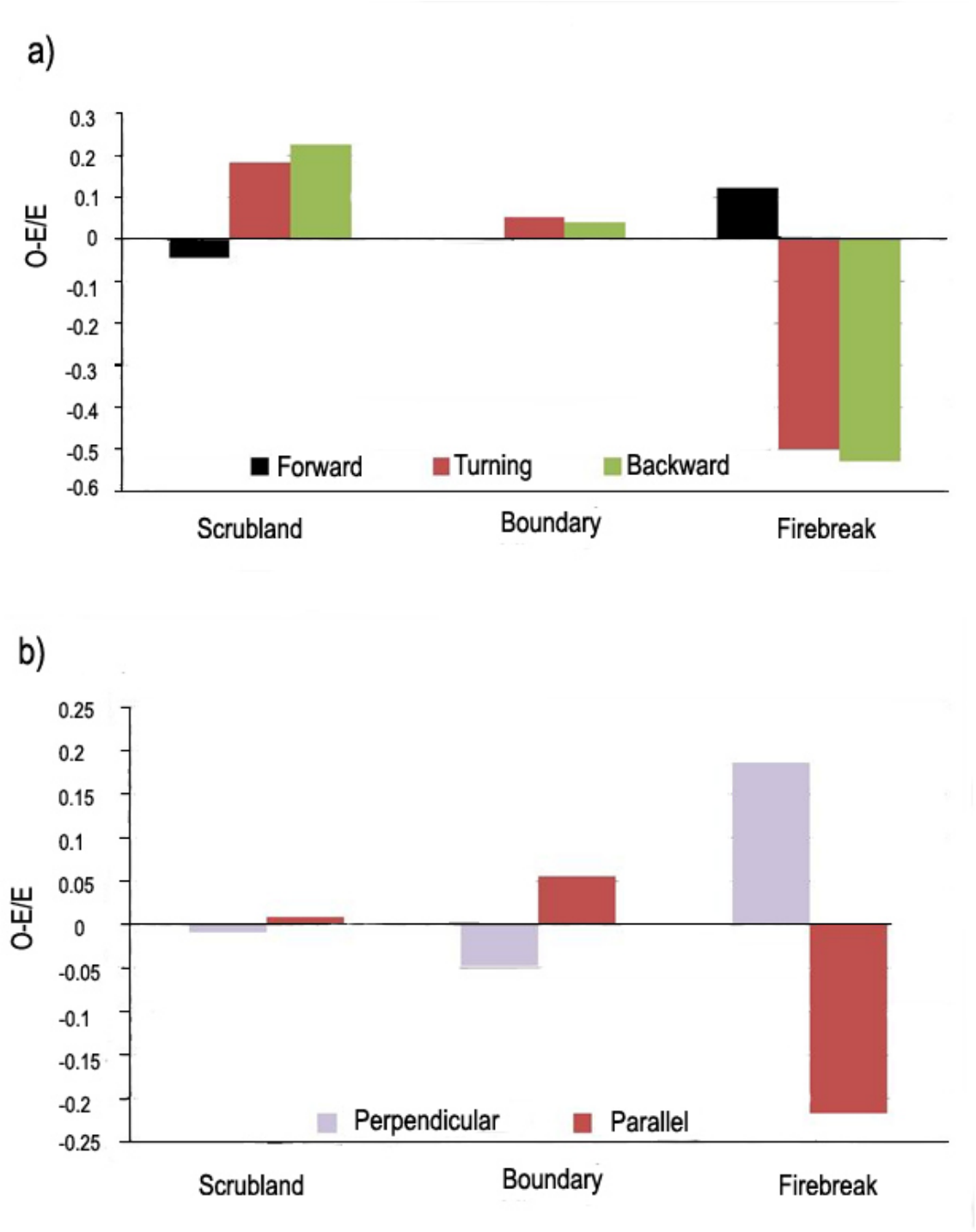

Fig. S2. Contribution to the chi-square statistic (O-E/E; O: observed frequency; E: expected frequency) of frequencies of categories of (a) turning angles and (b) azimuth in butterfly flights in scrubland, boundary and firebreak habitats

[CAPÍTULO 8:]

CONCLUSIONES

CONCLUSIONES GENERALES

Como conclusiones generales de este trabajo destacamos las siguientes:

Bloque I: La distribución espacial de *P. argus* a distintas escalas

1.-A escala de la Península Ibérica la presencia y abundancia de *P. argus* en el Parque Nacional de Doñana difícilmente puede explicarse en función de los requerimientos ambientales que esta especie tiene en sus poblaciones situadas más al norte. Los modelos de distribución muestran que es una población periférica aislada y ligada a la presencia de un acuífero muy superficial, que facilita la presencia de brezales, muy raros a esa latitud y altura sobre el nivel del mar.
Ecological Modelling (2015) 312: 335–346.

2.-A escala del Parque Nacional de Doñana la distribución de *P. argus* parece continua cuando se examina en cuadrículas de 1 Ha. No obstante, aparece una disminución de la abundancia desde el Este hacia las cuadrículas más elevadas y secas del Oeste de la zona estudiada. La estrecha relación mutualista que se establece entre las mariposas y la hormiga *L. niger* determina que ésta pueda utilizarse como un buen predictor de la presencia y abundancia de aquellas.
Ecological Applications (2005) 15: 3-18

3.-A escala local (decenas de metros) la distribución de *P. argus* está fuertemente condicionada por la topografía de las dunas estabilizadas por el matorral. La densidad de hormigueros y, como consecuencia la de plantas con huevos, tiende a aumentar hacia la parte media - baja de las laderas dunares, en zonas donde la humedad del suelo es mayor, pero sin llegar al encharcamiento.
Journal of Insect Conservation (2017) 21:873-883

4.-A escala de plantas nutricias las mariposas seleccionan ejemplares de *H. halimifolium* en función de la presencia de nidos de *L. niger* establecidos en su base y de una biomasa aérea que proporcione un microclima adecuado para el establecimiento de los hormigueros y para la alimentación de las larvas.
SHILAP Revta. lepid.,(2016) 44 (174) : 319-331

Bloque II: El movimiento de las mariposas

5.- El seguimiento de individuos mediante GPS es una técnica original en el estudio de los movimientos de mariposas relativamente sedentarias, de vuelo a baja altura y territorios llanos. Nuestros resultados ofrecen mucho mayor detalle que las técnicas tradicionales al localizar con precisión la posición de los individuos en una ventana de 1 segundo.
Journal of Insect Behavior (2016) 29:80–98

6.- Las mariposas se mueven en áreas relativamente pequeñas (1 Ha aproximadamente), donde abundan y coinciden los recursos de larvas y adultos. Son, por tanto, bastante fieles a los rodales donde nacen, en los que encuentran todos los recursos que necesitan.
Journal of Insect Behavior (2016) 29:80–98

7.-Los movimientos de la especie responden a la heterogeneidad del paisaje y el rango de vuelo medido permite la conexión entre distintos fragmentos de hábitat. Las trayectorias son muy distintas según el hábitat en el que se desplazan: movimientos más rápidos y rectilíneos en hábitats más hostiles y más sinuosos y lentos en hábitats más favorables.
Los cortafuegos no actúan como barreras, aunque si como filtros, puesto que se aprecia un elevado grado de rechazo al cruce.
Journal of Insect Behavior (2016) 29:80–98
Animal Conservation (en revisión)

8.- Eventuales descensos en el nivel freático llevan implícitos modificaciones en la estructura y composición de la vegetación y, como consecuencia, en la fauna de insectos que dependen de ella (hormigas y mariposas). Aunque la persistencia de *P. argus* en Doñana no es preocupante si se mantienen las condiciones actuales, el aislamiento de esta población impediría la recolonización desde otras áreas de la península.
Ecological Modelling (2015) 312, 335–346.
Journal of Insect Conservation (2017) 21:873-883
Animal Conservation (en revisión)

Agradecimientos

Es difícil saber el momento justo en que empieza una obsesión. Por mucho que vuelvas atrás y te remontes en el tiempo, no siempre está claro ese momento justo en que la curiosidad infantil se convierte en pasión y más tarde en obsesión. No hablo de la ciencia, hablo de los bichos. Pobres mariposas, hormigas y parasitoides que aguantaron mis incordios. Pobre conejos en cuarentenas, rapaces en el consultorio y linces en cautividad…. Pero sobre todo hablo de toda una familia peluda y plumífera que me ha acompañado con su fidelidad todos estos años, Lula, Cloe, Piña, Pitu, Chucky, Mili y Tonton, Kika, Linda, Pati, Rodolfo, Kaka, Kakito, Luka, Trufa, Piñon, Brezo, Brisa, Drito, Carchuno, Mofi, Jaspe, Cue cue, Negrin, Isidoro, Pantuflo, China, Siki, Ojitos-Wiso, Sancha, Pancho, Pepe y Panchita y Chikilicuatre. Todos culpables de mantenerme entretenida en un mundo más básico. Los que me conocen saben de su importancia en mi vida y tenían que tener su huequito aquí.

Este trabajo ha pasado de ser una obligación a ser un hobby más en el que la presión disminuyó cuando empecé a publicar los capítulos sin pensar en tesis ni nada por el estilo. La tardanza ha sido mucha pero en el camino he aprendido sobre todo a dar vueltas a tablas interminables de datos y jugar con ellos hasta llegar a cosas que ni siquiera estaban en el pensamiento. Esta tesis y el trabajo que conlleva me han enseñado a respetar aun más si cabe a los seres que nos rodean, a respetar su camino, lo entienda o no. Hasta una pequeña mariposa toma sus decisiones para vivir de la mejor manera posible

En tantos años de profesión seguramente olvidaré a personas que han participado de una y otra forma en el proceso largo que ha supuesto. En un principio como medio becaria o entusiasta voluntaria mil gracias al personal de la reserva biológica de Doñana y del palacio: Chans, Miguel Ángel, Rafa Laffite, Fabiola, Rosa, Manolo y cuantos soportaron las intrigantes maniobras con huevos y mariposas.

En el departamento no puedo olvidar a David Gutierrez que llegó en el momento clave para no abandonar sin siquiera haber empezado. Gracias a su claridad de ideas y su ayuda estadística fue posible plantear este trabajo, amen de las horas de campo que durante dos años compartimos. Si algo me llevo de este trabajo es su amistad que aún sigue fuerte. Junto a él, Adrian y Paqui, hicieron posible que 3000 mariposas se llevaran puestos unos cuantos lunares más. Y desde luego animaron la estancia con nuestras peleas caseras. Y los múltiples compañeros de café y campo, Joaquín, Rafa Villar, Emilio, Rafa Obregón, Salva, Laura, Sergio, Vico, Fran, Ana, Carmela, David, Manolo, Sole y sobre todo en los últimos tiempos, Bárbara, otra de las que me llevo en la mochila espero que para mucho tiempo. No puedo olvidar a Virtu, por ella me enganché en el departamento y las mariposas estas, por nuestras risas

compartidas pase los primeros años quizás aprendiendo más que nada lo que vale una buena amistad, sobre todo en los momentos malos.

A Juan y Diego por aguantarme pese a mis continuas escapadas a la caza de trabajo fijeo (una profesión en sí). A pesar de tantos años fuera trabajando en temas lejanos las sucesivas vueltas no han sido tan traumáticas como pudieron haber sido. Juan me trajo de vuelta en la prehistoria para trabajar con las plantitas de la Beña y continuar con este trabajo mariposero que ya estaba en el recuerdo y con Diego descubrí las posibilidades de la técnica. JFH, ha sido director de esta tesis hasta que "lo jubilé". A él realmente le debo este trabajo, me ha acompañado desde el principio y aun hoy sigue siendo algo más que un director de tesis.

Alejandro Rodríguez desde el otro lado del teléfono en Sevilla se convirtió en un apoyo inesperado.

Entre medias de todo, durante los años de trabajo en Doñana, el personal del Parque Nacional de Doñana, Blanca, Justo, Carlos, Mª Angeles, y Rafa Cadenas y aun mas especialmente Paco Robles, Celia y Pablo me hicieron ver la plasticidad que todos tenemos para adaptarnos a diferentes objetivos de vida.

Hace años, Celia Sánchez confió en mi para su proyecto de conservación de lince en Doñana y me dio la oportunidad de compaginar biología y veterinaria, además de llevarme al final a tomar la decisión más dura de cuantas he tenido, llevándole la contraria a todos cuantos me aconsejaron. Espero no tener que dales nunca la razón. Pablo Pereira me ha demostrado lo importante que es creer en uno mismo y la capacidad que todos tenemos para reinventarnos con éxito a nosotros mismos. Justamente en ese proceso de reinvención estoy, con abejas esta vez.

Entre medias de todo este ciclo de vida los compañeros de trabajo de Jaén, Elena, Rafa y Javier Moro entre muchos, confiaron de nuevo en mí para el trabajo con conejos y linces y me permitieron seguir trabajando en donde me encuentro más a gusto, que es el campo y sus acompañantes.

Desde que se cruzaron linces y conejos en la trayectoria de las mariposas ya todo se complicó. Todo se convirtió en ir saliendo de informes y trabajos de campo, necropsias de ciervos, gamos, jabalíes, conejos, seguimientos de mariposas, fototrampeo de linces, plantas en la breña, cuenta de cagarruteros de conejos, informes de vegetación, informes de linces y conejos. En definitiva, había que resolver de la mejor manera posible. Terminar la tesis queda en cierta manera en *stand by*. Sobre todo si valoras demasiado el tiempo que tenemos como para pasarlo demasiado atado a una silla.

A mi familia por aguantarme, pese a pensar en mas de una (y dos) ocasiones que esto no es normal, que ya es hora de sentar la cabeza y madurar. A ‘mamma’ Transi por aguantar cuando chica botes con orugas por todos lados, plantas y moscas en la nevera, hormigas en tubitos, conejos en el cuarto de baño, etc, etc, etc. Aparte de todas mis otras aficiones animadas.
Y finalmente a Paco que estuvo siempre por ahí detrás, sin entender mucho que hay en mi cabeza, y al final aceptando a los bichos casi más que a mí.